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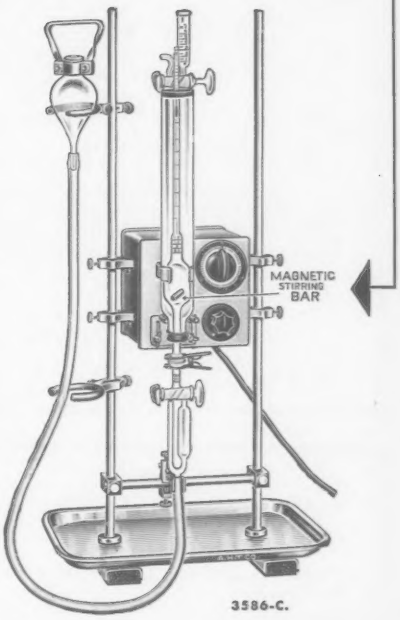

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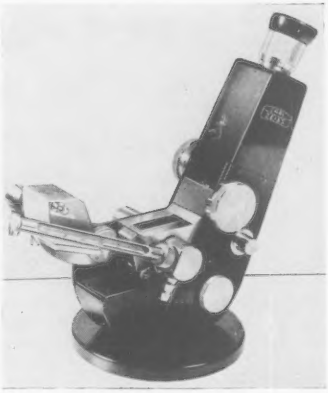

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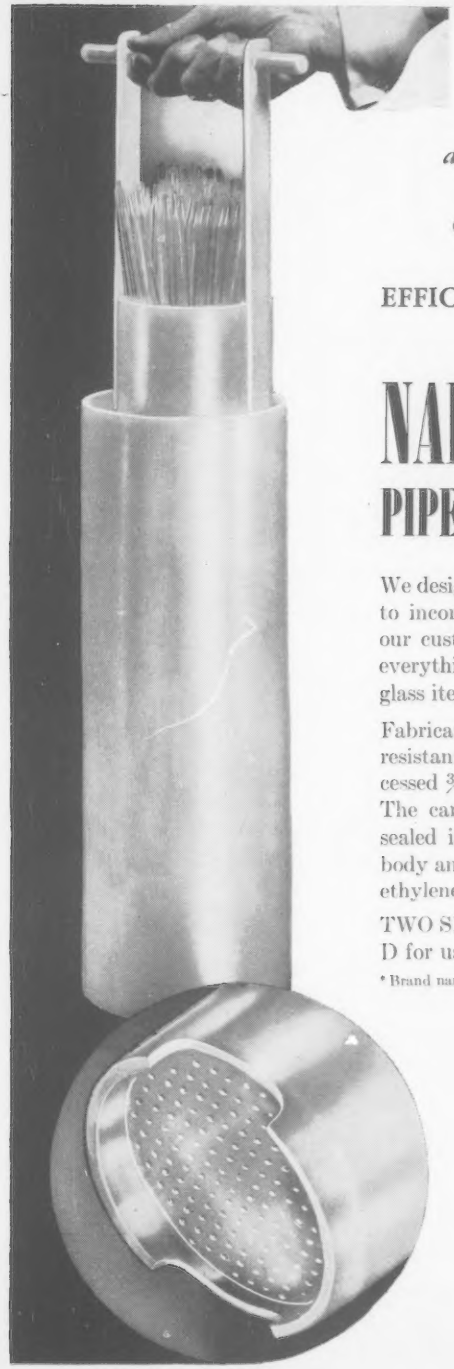

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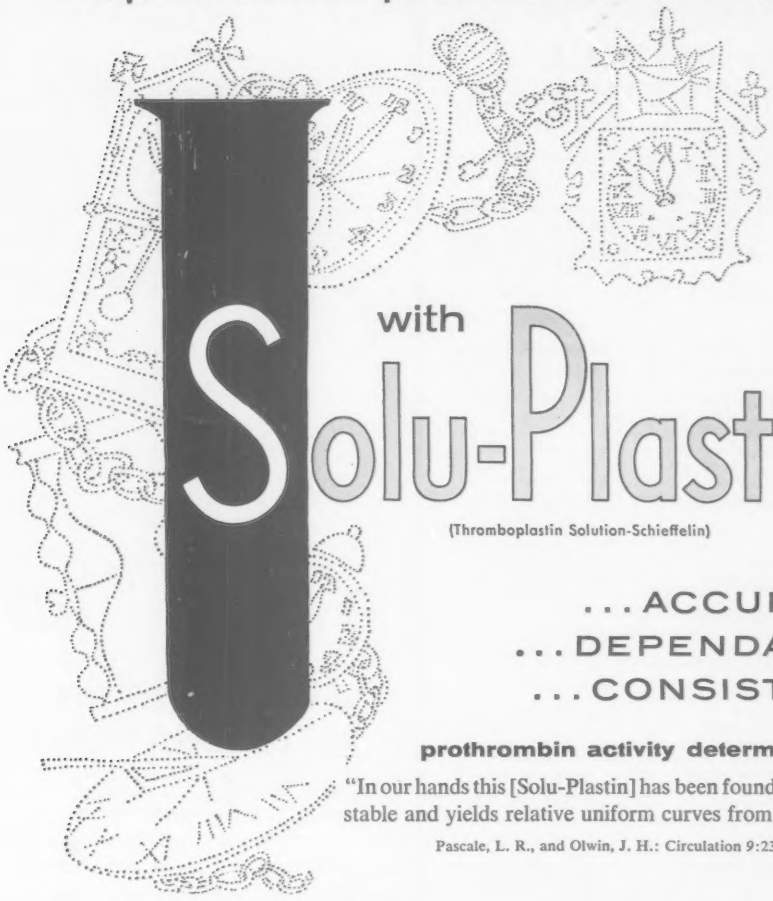

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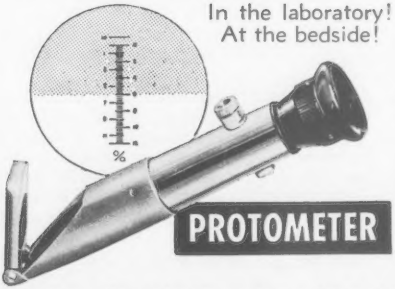

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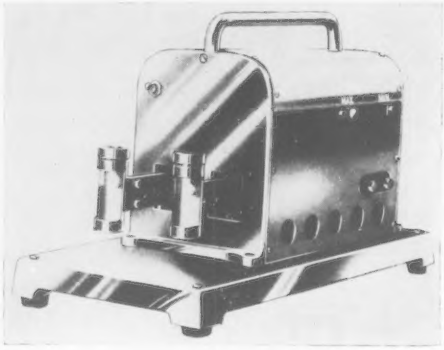

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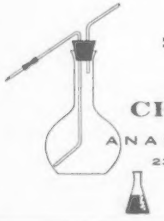

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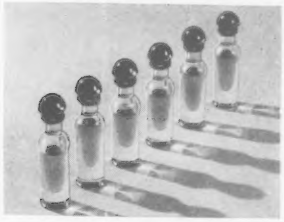

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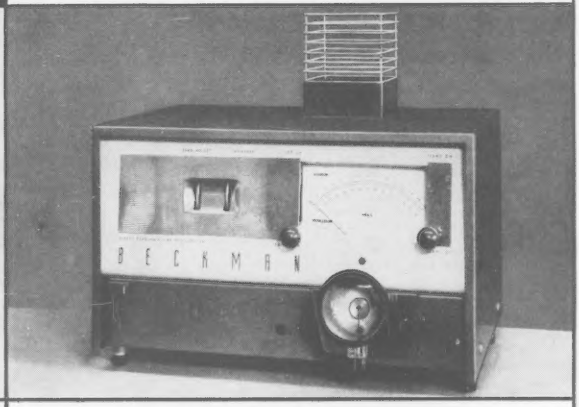

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Proposal for the Distribution of a Certified Standard for Use in Hemoglobinometry

Prepared by the Division of Medical Sciences, National Academy of Sciences-National Research Council

R. Keith Cannan

THERE IS PROBABLY NO PROCEDURE more commonly used in clinical laboratories than hemoglobinometry and few that are less satisfactory in their results. The deficiencies may reside, in part, in inadequacies of the chosen method. In larger part, however, they result from manipulative errors in the measurement and processing of the samples of blood. The latter involve questions of technical proficiency which are entirely within the control of the analyst himself. When, however, he converts his observations—gasometric or photometric readings, measurements of specific gravity, etc.—to grams of hemoglobin in 100 ml. of blood, he must depend upon his own calibration or a calibration supplied by an instrument maker, both procedures involving a conversion factor taken from the literature. To be satisfied that the calibration remains valid for the instrument, reagents, and technical routines in use in his laboratory, he should periodically undertake a precise and time-consuming series of standardizations. If a uniform hemoglobin standard were available nationally and a single method of analysis widely practiced, the individual laboratory would then have assurance not only that its results would be comparable from month to month, but that they would also be comparable with those from other laboratories employing the same standard. Inconsistencies in results that persisted under these conditions could then be clearly attributed to technical and manipulative errors.

Chairman, Division of Medical Sciences, National Academy of Sciences-National Research Council.

Presented in part at the meeting of the American Association of Blood Banks, Washington, D. C., September, 1954.

The purpose of this report is to describe a plan for the distribution of a certified hemoglobin standard and to advocate the wide adoption of a recommended analytic procedure. The use of both the new procedure and the standard is recommended. At the same time it is pointed out that the standard may be employed for the calibration of other methods of hemoglobin analysis in laboratories that do not choose to adopt routinely the advocated photometric procedure.

In 1941 the British Medical Research Council instituted an extensive study of the problem of standardizing hemoglobinometry and emerged with (a) a recommended analytic procedure, (b) a simple hemoglobinometer for general use, and (c) a certified national hemoglobin standard for distribution to cooperating laboratories (1, 2, 3, 4).

In the United States the initiative was taken by the Army Medical Service Graduate School with a limited field trial (5) of a cyanmethemoglobin solution proposed by Dr. David L. Drabkin. The success of the Army plan so impressed the Hematology Study Section of the National Institutes of Health that it requested the National Research Council to explore the possibility of establishing a National Hemoglobin Standard for general use throughout the country.

To this end the Academy-Research Council established an *ad hoc* panel under the Subcommittee on Blood and Related Problems of the Division of Medical Sciences. This panel has sought the cooperation of the College of American Pathologists, the American Society of Clinical Pathologists, the American Association of Blood Banks, the Department of Defense, the Veterans Administration, the National Institutes of Health, and the National Bureau of Standards. It has also maintained close liaison with the Committee on Hemoglobinometry of the Medical Research Council of the United Kingdom and with the National Research Council of Canada.

The panel gave serious consideration to the British plan, but decided that, in respect to simplicity and adaptability, the cyanmethemoglobin method (6, 7, 8, 9, 10, 11) adopted by the U. S. Army would be more suitable for use in the United States and Canada. It was agreed that the choice of a solution of some form of hemoglobin as a standard for hemoglobinometry was both logical and direct. In contrast to a glass standard, it would have the advantage of adaptability to a variety of photometric instruments and cuvettes. Among the forms of hemoglobin well adapted to photometry, cyanmethemoglobin has outstanding advantages. It has been shown that solutions of this pigment are stable for years when preserved at refrigerator temperatures (12). The absorption band of cyan-

methemoglobin in the region of 540 mμ is broad rather than sharp, so that its solutions are suitable for use in filter type photometers as well as in narrow band spectrophotometers (13, 7). Finally, all forms of hemoglobin likely to be found in blood, with the exception of sulfhemoglobin, are quantitatively converted to cyanmethemoglobin upon the addition of a single reagent (7).

RECOMMENDATIONS

On the basis of these considerations, the panel reached agreement on the following recommendations:

1. Standard of Reference

There shall be a Standard of Reference in the form of a preparation of crystalline human hemoglobin prepared by the method of Drabkin (14, 15, 16). The acceptable criteria for this preparation shall be that a solution containing 1 milliatom of hemoglobin iron per liter shall have a millimolar extinction coefficient of 11.5 at a wave length of 540 mμ, when measured as cyanmethemoglobin (13, 7). Certification of this Standard of Reference shall rest upon the results of spectrophotometric measurements and of analyses for iron made independently by the National Institutes of Health, the Army Medical Service Graduate School, the National Bureau of Standards, and Dr. Drabkin's laboratory at the University of Pennsylvania.

Professor King of the Postgraduate Medical School in London will also characterize the Standard of Reference both chemically and spectrophotometrically and compare the results of its use with determinations of hemoglobin employing the British standard.

2. Iron Content of Hemoglobin

The iron content of hemoglobin shall be accepted to be 0.335 per cent (17). This value for iron is the traditional figure used in this country and is in substantial agreement with that adopted by the British. It corresponds with an equivalent weight for hemoglobin of 16,700 per atom of iron and with an oxygen capacity of 1.34 ml. per gram of hemoglobin (17).

The adoption of an agreed figure for the iron content is necessary in order that the spectrophotometric measurements in terms of iron may be translated into grams of hemoglobin. Should any change be made in the future in the accepted values for the extinction coefficient of cyanmethemoglobin and the iron

content of hemoglobin, results based on the use of the above standard may be readily recalculated.

3. Standard for Distribution

There shall be a Standard for Distribution in the form of a certified solution of cyanmethemoglobin which shall be prepared directly from the Standard of Reference. The Standard for Distribution shall be packaged as three separate solutions containing certified concentrations of approximately 20, 40, and 60 milligrams of hemoglobin in the form of cyanmethemoglobin per 100 ml.

These three solutions will correspond to 1:250 dilutions of blood containing approximately 5, 10, and 15 Gm., respectively, of hemoglobin per 100 ml. After bottling, samples of these standard solutions will be spot-checked for correctness of optical density by the four analytic laboratories that have been designated above. The batch will then be certified and distributed to cooperating laboratories by designated national agencies.

4. Adoption of Cyanmethemoglobin Method

In conjunction with the use of the proposed standards, it is recommended that clinical laboratories consider the adoption of the cyanmethemoglobin method of hemoglobin determination described by Drabkin.

The use of the cyanmethemoglobin method follows from the concept that it would be logical to adopt a method of analysis which converts hemoglobin into the same pigment as that used in the standard. It does not, however, preclude the use of the cyanmethemoglobin standard for the calibration of other methods of hemoglobin analysis which may be in routine use in some laboratories. However, it should be realized that such a procedure may lead to some loss of accuracy.

The cyanmethemoglobin method employs a single solution containing potassium ferricyanide and potassium cyanide, which converts the hemoglobin in blood quantitatively to cyanmethemoglobin. The ferricyanide converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with potassium cyanide to produce the stable pigment cyanmethemoglobin. These two reactions are rapid and stoichiometric.

There should be no reluctance to employ this standard and reagent because they contain cyanide. The concentration of cyanide in the reagent that is proposed for use is only 52 mg. of potassium cyanide per liter. Its lethal dose for man approaches four liters (18). Most clinical laboratories use for the determination of uric acid, a reagent containing 50 Gm. of this salt per liter. In view of this and of the fact that laboratories of clinical pathology are disciplined in the use of such dangerous materials as isotopes and virulent pathogens, it would

seem that the handling of the proposed reagent constitutes a quite negligible hazard.

The panel also agreed to undertake a field trial of one year's duration using the Standard for Distribution described above. It is now felt that the plans for this field trial have progressed to the point where the participation of laboratories desiring to cooperate may be invited. The standards will be prepared by Dr. David L. Drabkin and distributed without charge to clinical laboratories on application, provided they will agree to meet certain minimum requirements for participation, as follows:

1. To conduct and report at three-month intervals, for one year, measurements of the actual photometric readings of the three standard solutions in the photometer routinely in use for hemoglobin measurements in that laboratory.
2. To cooperate in answering a simple questionnaire designed to furnish information on the influence of various factors on the results of the hemoglobin determinations which will assist the panel in its long range plans for making this standard available on a national scale.
3. To cooperate in the analysis and reporting of (a) an unknown solution of cyanmethemoglobin, and (b) an unknown sample of blood.

The Standard for Distribution, consisting of the three solutions described above, will be packaged as a single unit. Details of the procedure for the determination of hemoglobin as cyanmethemoglobin, as well as details of the procedure for calibrating another method in terms of the cyanmethemoglobin standard, will be furnished with the standard.

Distribution will be made to civilian laboratories by the College of American Pathologists, 203 North Wabash Avenue, Chicago, Illinois; to military and government laboratories by the Army Medical Service Graduate School, the Navy Bureau of Medicine and Surgery, the Air Force Surgeon General's Office, and the Veterans Administration* and to laboratories in Canada through the Division on Medical Research, National Research Council, Ottawa, Ontario, Canada. Cooperating laboratories are requested to apply to the distributing agency with which they are most closely associated. Because of limitation in the number of sets of the standard available, distribution will be determined by priority of application and willingness to comply with the conditions listed above. Application for standards will assume acceptance of these conditions. It is estimated that the Standard will be ready for distribution by April 15, 1955.

* Veterans Administration, Central Office, Room 871.

This plan has been drafted by the *ad hoc* Panel on the Establishment of a Hemoglobin Standard of the Division of Medical Sciences, National Academy of Sciences-National Research Council.

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Rapid Estimation of Amylase Activity of Serum by Turbidimetry

Oscar Peralta and John G. Reinhold*

THE CHOICE OF RAPID METHODS for estimation of serum amylase activity is limited (1). Such methods depend upon the change in color of starch with iodine brought about by splitting of the starch by the enzyme. It is often difficult to establish an end point visually by this reaction in partially digested starch solutions, especially by those whose experience with the method is limited. Furthermore, the use of solutions of elemental iodine in laboratories in which microanalytical methods for iodine are applied introduces a considerable hazard of contamination to the latter.

Favorable experience with the turbidimetric method of Waldron for measuring the amylase activity of pancreatic juice suggested that this procedure might be adapted to the rapid estimation of amylase activity of serum. The present paper describes such a method and offers evidence that it is capable of detecting elevation of amylase activity in serum.

This approach to the estimation of amylase activity of serum has received little attention. A turbidimetric method for measurement of serum amylase activity was described by Jacobsson and Hansen, who used dextrin as a substrate. Their method called for a 30-minute period of incubation and removal of protein before turbidimetric measurement of dextrin concentration. The method to be described requires less time

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The authors wish to acknowledge the assistance in various ways of the following in the development and trial of the method described: Misses Lola Allen, Sonya Graboyes, Edythe Gershman, Shirley Matis, Joan McNichol, Doris Thompson, Ruth Winger, and Dr. Frank Dituri.

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and fewer manipulations than that of the Scandinavian workers. Another method of applying turbidimetry to estimation of amylase activity of pancreatic secretion is that of Guth, Komarov, and Shay.

The basis of the turbidimetric method for measurement of amylase activity is the decrease in the absorbency of a buffered starch suspension caused by the splitting of the starch by the enzyme during a timed interval and at a controlled temperature.

REAGENTS

Stock Starch Suspensions

Fifty grams of acid-digested cornstarch (Argo Laundry Starch has proved satisfactory) are crushed to a powder (in a mortar, if necessary). A preservative (5) of 1.5 Gm. of methylparahydroxybenzoate and 0.3 Gm. of propylparahydroxymethylbenzoate may be mixed with the starch powder, although it is not essential. Water, 150 ml., is added and the resulting thin paste poured slowly into 850 ml. of boiling water in a beaker while stirring rapidly with a mechanical stirrer. The mixture is heated just to boiling on an asbestos-covered electric hotplate, the stirring being continued. 10 Gm. of sodium chloride are added. When cool, the starch suspension is transferred to a wide-mouth bottle and stored, well stoppered, at room temperature.

If the starch suspension thickens, aliquots for preparation of diluted starch suspension are removed by means of a spoon or spatula.

A more convenient source of substrate is the liquid starch suspension widely distributed under the trade name "Zippy." Standardized preparations of this product contain 10% starch and are without the blue dye present in starch sold for household use.[1] The pine oil included as a preservative does not interfere. Although the blue dye present in household starch does not demonstrably affect the action of amylase, and acceptable results may be obtained with liquid laundry starch as purchased in the market, the dye complicates the standardization of the substrate and dye-free preparations are preferred.

Diluted Starch Suspension

Sufficient stock starch suspension is added to the buffered NaCl diluent in a stoppered cylinder to give a reading of approximately 325

[1] Available from the Hartman-Leddon Company, Philadelphia, Pa.

scale divisions on a Klett-Summerson photocolorimeter or a transmittance of 25 per cent if other photoelectric photometers are used. Usually a dilution of 1 part of stock starch suspension to 10 parts of buffered NaCl diluent approximates the desired concentration. The diluted starch suspensions remain usable for as long as 5 days. The starch always must be completely dispersed by thorough mixing before use.

Buffered Sodium Chloride Diluent

13.0 Gm. of NaCl, 5.9 Gm. of KH_2PO_4, and 15.9 Gm. of Na_2HPO_4 (anhyd.) are dissolved in water and diluted to 1000 ml. The *p*H is 7.2.

METHOD

All tests are done in duplicate.

1. Measure 5 ml. of diluted starch suspension into a cuvette and place it in a water bath at 38° for approximately 5 minutes.
2. Measure 1 ml. of serum into a tube of about 15 ml. capacity. Mix the warm starch suspension and serum by pouring the starch into the serum and then pouring back and forth 2 times before returning the mixture to the cuvette. Start a timer, which has been set at 5.5 minutes, immediately after the starch and serum have been mixed. Adjust the zero setting of the photometer by means of a cuvette containing distilled water.
3. Exactly 0.5 minutes after mixing, measure the absorbency (A_1). Replace the cuvette in the water bath.
4. Exactly 5 minutes later, mix and again measure the absorbency (A_2). Readjust the zero setting if necessary before making the reading.

CALCULATION

Convert A_1 and A_2 into percentages by means of the standard curve. The amylase activity is expressed as a velocity constant, k.

$$k = \frac{1}{5} \times 2.3 \log \frac{C_1}{C_2} \times 10{,}000 = 0.46 \log \frac{C_1}{C_2} \times 10{,}000$$

C_1 and C_2 are the initial and final concentrations expressed as percentages. To obtain whole numbers, k is multiplied by 10,000. Table 1 shows k values corresponding to the various ratios of C_1/C_2.

STANDARDIZATION

Measure the volumes of buffered NaCl solution and diluted starch suspension shown in Table 2 into cuvettes of uniform diameter. Place

Table 1. VALUES OF "k" CORRESPONDING TO VARIOUS FINAL CONCENTRATIONS OF STARCH SUBSTRATE

Initial concentration = 100 per cent

Final concentration (%)	k ($\times 10^4$)
99	20
98	40
97	61
96	82
95	103
94	124
93	145
92	166
91	188
90	210
89	232
88	255
87	277
86	300
85	324
84	349
83	373
82	397
81	422
80	448
79	472
78	496
77	522
76	548
75	574
74	601
73	628
72	655
71	682
70	712
69	741
68	770
67	800
66	830
65	860

If the initial reading does not correspond to 100 per cent on the standard curve, plot the initial absorbency of the diluted starch suspension (plus serum) on the abscissa corresponding to 100 per cent. Then draw a line parallel to that given by the 3 standards. Convert the absorbency of A_2 to percentage by means of the new line.

Percentages between those shown in the table may be converted to k values by interpolation.

Table 2. VOLUMES FOR STANDARDIZATION

Standard No.	*Buffered NaCl* (ml.)	*Diluted starch suspension* (ml.)	*Per cent of initial concentration*
1	1	5	100
2	2	4	80
3	3	3	60

the cuvettes in the water bath at 38° for 5 minutes. Mix the contents thoroughly and measure the absorbency. Plot absorbencies against percentages. The line obtained is nearly straight.

HIGH AMYLASE ACTIVITIES

The quantity of starch provided in the substrate is not sufficient to enable accurate measurement of high amylase activities. If the change in absorbency exceeds 0.12 (60 scale divisions on the Klett-Summerson photocolorimeter), the test should be repeated, using 0.5 ml. of serum or less.

Micro Method

Serum samples of 0.2 ml. are combined with 1.0 ml. of diluted starch suspension and the absorbencies are measured in micro cuvettes. Smaller samples may be used if appropriate cuvettes are available.

COMMENTS

Studies of amylopsin and of serum amylase showed that starch suspensions cleared more rapidly when the enzyme acted at 38° than at the lower temperature used by Waldron. Since the main purpose of introducing the new method was to shorten the time required for estimation of amylase activity, the higher temperature was adopted.

The activity of amylase is favored also by high concentrations of substrate and it diminishes rapidly as the concentration of substrate decreases. It is advantageous, therefore, to use a short time interval, and the 5-minute reaction time serves not only to expedite the analysis but makes possible a more sensitive measurement of enzyme activities than would be obtained by longer incubation times.

The buffer solution is that described by Guth, Komarov, and Shay. The *p*H is not critical. Measurements are made at 660 mμ so as to enable maximal concentrations of starch to be used while still making possible measurements within a favorable absorbency range of the photometer. This wave length also avoids interference by pigments of serum and diminishes the contribution of turbidity in the serum.

RESULTS

The results obtained by the rapid turbidimetric method have been compared with those of the Somogyi saccharogenic method (6). Reducing substances were measured by the Nelson-Somogyi method (7, 8), as described by one of the authors (9). Figure 1 shows that speci-

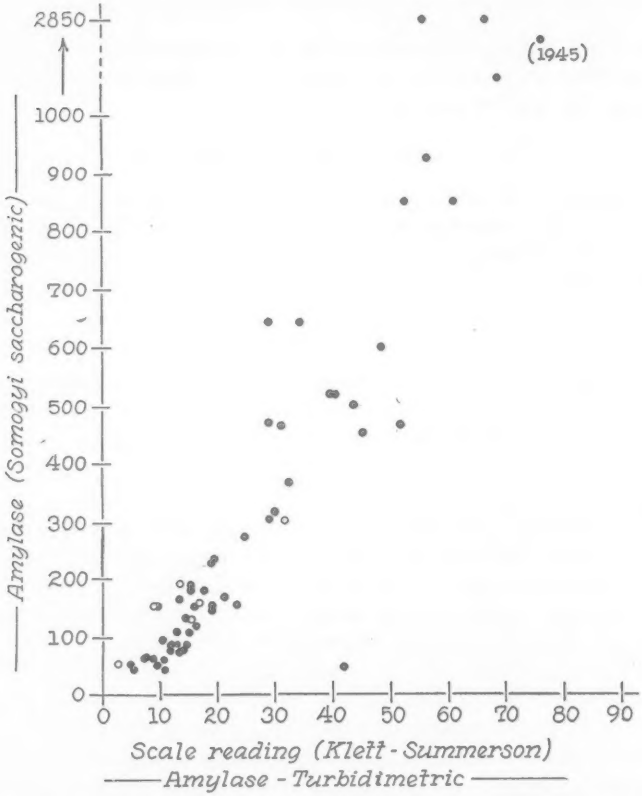

Fig. 1. The correlation between amylase estimated by the turbidimetric method and the Somogyi saccharogenic method. Where symbols are paired, each represents a single determination. Open circles show the results of determinations made by the micro technic, using 0.2 ml. of serum and 1 ml. of diluted starch suspension. All the abnormal values, with one exception (see text), were associated with pancreatic disease and are clearly differentiated from those of the control group.

Figures on the abscissa are scale readings of the Klett-Summerson photocolorimeter; those on the ordinate as glucose, in mg./100 ml.

mens having high activities by the standard method also had significantly increased values by the turbidimetric method. However, those having excessively high activities did not show a proportionate increase by the turbidimetric method unless smaller amounts of serum—0.5 ml. or 0.05 ml. for macro and micro techniques, respectively—were used.

Activities of serum amylase have been measured in 41 healthy individuals by use of the turbidimetric method. These included laboratory workers, staff physicians, and blood donors. The distribution of values is shown in Fig. 2.

The turbidimetric method has been applied to serum during the past 6 months in the laboratories of the authors, especially when emergencies required prompt reports. In only 1 instance out of approximately 50 trials did the results differ from those of the standard Somogyi method. The exception was attributed not to a failure of the method but to a technical error by an intern working alone at night, who lacked actual experience with the method. A recurrence of this error was traced to contamination of the serum by blood cells.

SUMMARY

The method of Waldron for turbidimetric measurement of amylase activity of duodenal contents has been adapted to measurement of

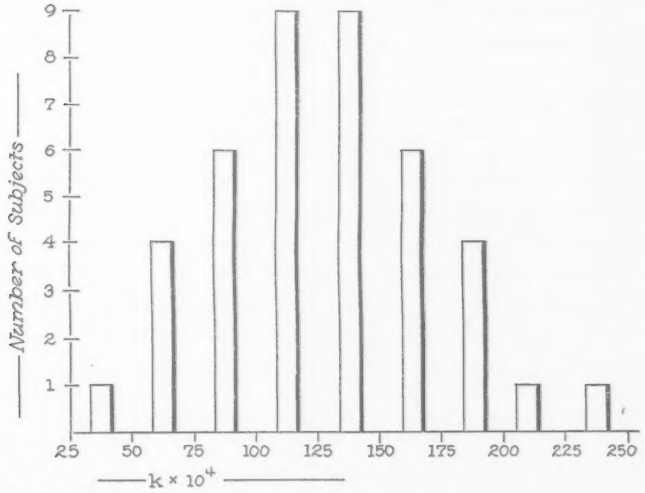

Fig. 2. Distribution of k ($\times 10^4$) values of a group of 41 healthy control subjects whose serum amylase activities were measured by the turbidimetric method.

amylase activity of serum. The speed and simplicity of the method make it suitable particularly when results are needed quickly.

Comparison of the results obtained by the turbidimetric method with those of a saccharogenic method showed that high amylase activities could be established dependably by the turbidimetric method.

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Improved Methods of Analysis for Oxygen, Carbon Monoxide, and Iron on Fingertip Blood

Samuel Natelson and Carol M. Menning

THE TENDENCY OF CERTAIN premature and new born infants to become cyanotic necessitates a rapid and convenient method for the determination of oxygen, iron, and carbon monoxide on minute quantities of blood. These procedures are necessary to distinguish between cardiac anomalies, methemoglobinemia, lung abnormalities, and other conditions that result in anoxia to tissues (1–6).

The procedures described herein for oxygen and carbon monoxide use an improved form of a microgasometer, described previously (7, 8), in which both gases are estimated manometrically. A photograph of the instrument with an inset showing the method of sampling is shown in Fig. 1.

For the estimation of oxygen in blood the usual technic for liberating the O_2 by ferricyanide, removing the CO_2 with alkali, and absorbing the oxygen in sodium hydrosulfite is used (9–11). For carbon monoxide the same steps are followed using a special reaction chamber (Fig. 2). The CO is absorbed in cuprous chloride–ammonia complex (12, 13).

Iron is estimated as the thiocyanate by a modification of a method previously described (8) after wet ashing the blood (14). The use of small quantities of blood permits the use of relatively large amounts of perchloric and sulfuric acids so that digestion is rapid

From the Department of Biochemistry of the Laboratories of Rockford Memorial Hospital, Rockford, Illinois.

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REAGENTS AND METHODS

Estimation of Oxygen Capacity and Content

Reagents

Simultaneous deaeration of reagents is carried out for approximately 10 minutes just before use, by transferring the reagents to small containers (Fig. 3). Reduced pressure is obtained by means of a water aspirator and a glass manifold. As the reagents are layered with oil, they remain air-free. During the 10-minute deaeration process, the bottles or vials are tapped occasionally to facilitate the displacement of air.

Potassium Ferricyanide: 1.2% in distilled water. Transfer a portion of this solution to a 20 ml. short form vial and cover with a layer of caprylic alcohol. After deaeration the reagent is removed for the procedure by taking up, in order, caprylic alcohol, the reagent, and then caprylic alcohol. Contamination of this reagent with mercury should be avoided.

Saponin Solution: 0.5% in distilled water. Transfer a portion to a 20 ml. vial, cover with mineral oil, and add mercury to about 2 cm. high in vial. Deaerate just before use.

Potassium Hydroxide: 1N. Keep in a polyethylene bottle. Transfer a portion to a 20 ml. vial and cover with mineral oil. Mercury is added to a height of 2 cm. in vial.

Sodium Hydrosulfite Solution: Place 1 Gm. sodium hydrosulfite placed in a vial or test tube and cover with mineral oil. Add 5 ml. of 1N KOH and 2 ml. of mercury. The powder will dissolve as the solution is being deaerated. If not completely dissolved, deaerate while tube is in a 60° water bath. It is best that this solution be prepared daily.

Preparation of Microgasometer

Draw approximately 1 ml. of water into the 3 ml. bulb and expel. Repeat. Draw 0.1 ml. of 1N lactic acid into the instrument, retreat to the 3 ml. mark, and then expel the acid. The gasometer is thus left acid which eliminates the need for an acid ferricyanide reagent.

Collection of Sample

Prick the fingertip or heel and allow the blood to flow into a heparin-lined tube, 1.5 mm. bore, as described previously (7, 8). Discard the first drop by wiping with a cotton pledget, insert the tip of the fire-polished tube into the cut, and allow the blood to flow directly into the tube until approximately 10 cm. (0.1 ml. approx.) has been collected.

Oxygen Capacity: Transfer the blood to the bottom of a silicone-lined,

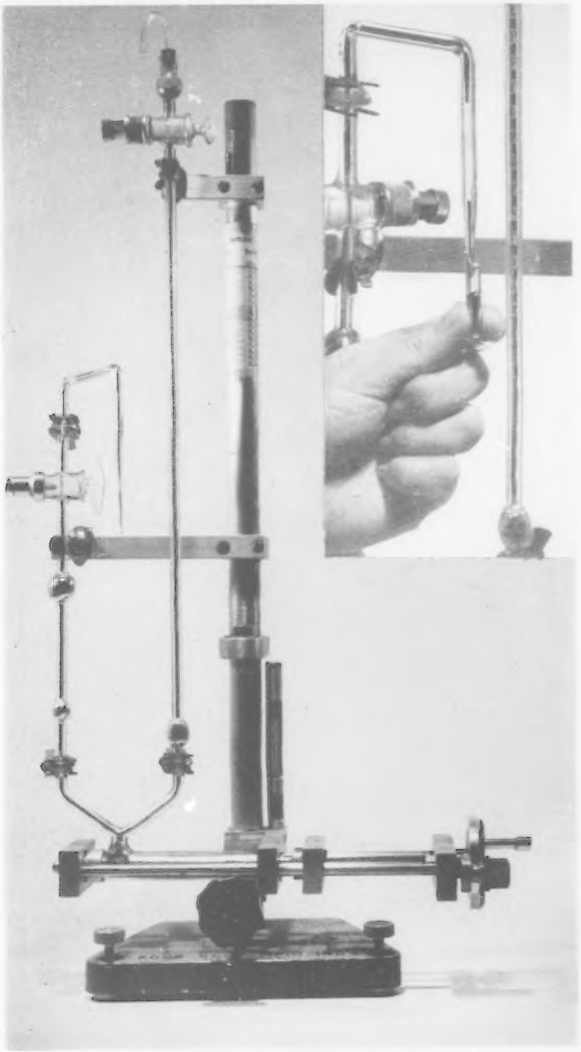

Fig. 1. Microgasometer showing method of sampling in inset. Positioning of the mercury is accomplished by rotating the wheel.

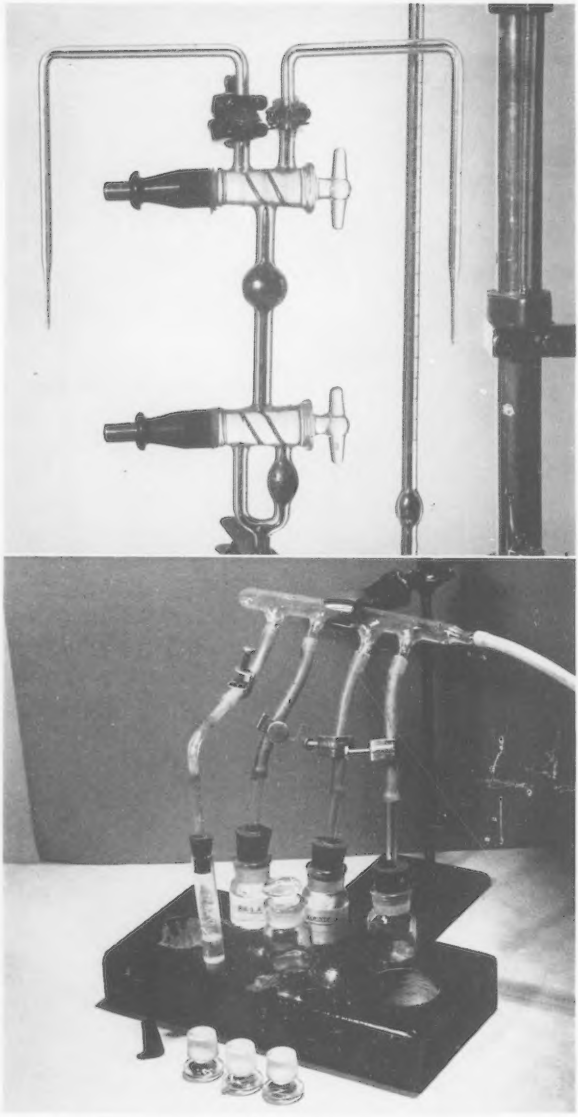

Fig. 2. Reaction chamber for CO estimation. Two-way stopcock at top permits rinsing of the instrument while the gas bubble is trapped at right above stopcock. Lower stopcock is not used in this determination.

Fig. 3. Simultaneous deaeration of reagents for oxygen and carbon monoxide estimation with the water aspirator.

3 ml. test tube (10 × 75 mm.) that has been cut to half its height. The blood is saturated with oxygen by beating the tube with the fingers, as for shaking, for about three minutes. As an alternative, a slow stream of O_2 or air is passed through the blood from a fine-tipped glass tube. Just before performing the analysis the blood is again shaken to resuspend the cells. Approximately 0.05 ml. is transferred to a 0.5 ml. tube half filled with mercury. This tube is made by cutting in half the 1 ml. test tube (6 × 50 mm.). An additional tube and sample should be used for duplicate determinations.

Oxygen Content: This is best done if the gasometer, which is portable, is placed within a few minutes walking distance of the patient. Collect the blood in the same manner as for O_2 capacity and transfer about 0.05 ml. directly into the 0.5 heparinized tube containing mercury covered with a layer of mineral oil. Insert the collecting tube so that its tip extends below the mineral oil and allow the blood to layer under it, leaving some in the collecting tube. The blood sample has not been exposed to air except for the minute surface at the narrow collecting tip. For the duplicate, wipe the tip of the collecting tube and layer another sample. The collecting tube should rest in a horizontal position so that subsequent sampling is representative, the cells settling laterally in the tube. If settling is marked, the tube may be rolled on the table to resuspend the cells. When both determinations are to be done on the same sample it is best to do the O_2 content first using the remaining blood for the O_2 capacity.

Procedure

Sampling of the reagents into the gasometer is done by advancing the mercury so that a bubble protrudes from the end of the capillary tip, dipping the capillary tip into the sample to be taken up, retreating with the plunger until the required amount has been sampled, and then following the sample with mercury or caprylic alcohol without withdrawing from the solution. The procedure is necessary to exclude air.

First, 0.03 ml. of whole blood is drawn up followed by a mercury plug. Next 0.02 ml. caprylic alcohol, 0.03 ml. of ferricyanide reagent, and 0.02 ml. caprylic alcohol are drawn up in that order. Finally, 0.1 ml. saponin solution is taken into the instrument followed by mercury until the sample and all the reagents are drawn to the 0.12 mark beyond the stopcock, which is then closed. All reagents are drawn into the lower bulb by retreating with the plunger and mixed well. If any mercury has remained in the tube, it is removed by vigorous shaking with the shaking

rod or by advancing and allowing the bubble of released gas to get above the mercury and retreating to permit the mercury to drop down. The plunger is retracted until the mixture is below the 3 ml. mark and allowed to remain there for three minutes, with occasional shaking. To be certain that all O_2 has been released, a reading is taken on the manometer when the caprylic alcohol meniscus is at the 0.12 ml. mark. The mercury is lowered again and then brought back to check the reading. When two consecutive readings check, the O_2 liberation is complete.

The screw is advanced to release the pressure. The stopcock is opened and 0.03 ml. of 1N KOH is introduced followed by mercury to bring all the KOH beyond the stopcock, which is then closed. The plunger is retracted to the 3 ml. mark, the mixture shaken, and the caprylic alcohol meniscus brought back to the 0.12 ml. mark. The pressure on the manometer (P_1) is read.

The pressure is released and 0.03 ml. of hydrosulfite reagent is introduced, followed to the 0.12 ml. mark with mercury, and the stopcock closed. The reaction chamber is evacuated to the 3 ml. mark and the caprylic alcohol meniscus brought back to the 0.12 ml. mark. The pressure (P_2) is read. At this point the precipitated methemoglobin (brown) should turn to the red of hemoglobin. If this is not the case the hydrosulfite reagent is at fault. The analysis is not lost since 0.03 ml. of freshly prepared hydrosulfite may be added and the reading (P_2) taken.

Calculations

$$(P_1 - P_2) \times \text{factor (Table 4)} = O_2 \text{ in vols./100 ml.}$$

$$O_2 \text{ in vols./100 ml.} \times 0.446 = O_2 \text{ in mM/l.}$$

Estimation of Carbon Monoxide Saturation of Blood

Preparation of the Sample

The sample is collected as for oxygen capacity and placed in the silicone-lined test tube. A stream of CO (HOOD) is passed through the blood by means of a tube drawn to a fine tip. The CO is generated by dropping conc. H_2SO_4 on formic acid contained in a suction flask, by means of a dropping funnel and a one-holed rubber stopper. The gas is passed through a tube containing soda lime and then calcium chloride (anhyd.) to remove acids and water.

Reagents

The reagents are the same as for O_2 estimation with the following additional ones.

Cuprous Chloride-Ammonia Reagent: 15 grams of cuprous chloride is added to 25 Gm. of ammonium chloride and made to 100 ml. with water. 30 cm. of fine copper wire, which has been cleaned with sandpaper and thoroughly rinsed in distilled water, is added. The copper wire is used in short lengths. Excess copper wire must always be present. The mixture is covered with a layer of mineral oil. The solution is allowed to stand overnight, or warmed to 60° until the solution becomes colorless. Some excess cuprous chloride will remain at the bottom. Transfer some of the supernatant to a vial containing mercury and mineral oil and deaerate by suction. The original solution is stable indefinitely.

Ammonium Hydroxide Buffer: To 25 Gm. of ammonium chloride add 100 ml. of water and 50 ml. of concentrated ammonium hydroxide. Transfer some to a vial containing mercury.

Air-free Water: Add water to a bottle containing mercury and mineral oil and deaerate as above. This is used for washing and approximately 100 ml. of such water should be kept on hand.

Procedure

For CO estimation the cuprous chloride solution may not be mixed with the other reagents used for removal of O_2 and CO_2. For this reason, a two-way stopcock is needed. Figure 2 shows the reaction chamber used. The bottom two-way stopcock is not required for this determination. This was left open so that it led into the chamber below the stopcock.

The procedure for O_2 determination is followed exactly as described. However, the time required for complete release of CO is much longer than that for O_2. Thus, after addition of the ferricyanide, five minutes must be allowed for liberation of all the gases. After the oxygen has been removed, 0.1 ml. of caprylic alcohol is introduced into the instrument followed by mercury. The residual gases (CO, N, and the rare gases) are pushed up to the capillary tube on the right by turning the stopcock 180° and advancing the plunger until the gas bubble is pushed above the stopcock with a little caprylic alcohol acting as the seal. The pipet at the right contains mercury so that the gas is trapped between caprylic alcohol and mercury. The stopcock is turned to its original position and all the reagents are expelled. The machine is rinsed twice with air-free water and the mercury pushed to the tip of the pipet.

The bubble of gas is now returned to the instrument by rotating the stopcock and drawing the bubble followed by a little mercury into the instrument and again returning the stopcock to its original position. Then 0.1 ml. of ammonia buffer followed by 0.1 ml. of water is drawn

into the gasometer, followed by mercury until the reagents are beyond the stopcock. The stopcock is closed and evacuation made to the 3 ml. mark. Shake for one minute, bring to the 0.12 ml. mark, and read (P_3). The pressure is released by advancing the mercury. The stopcock is opened and 0.03 ml. of the cuprous chloride reagent followed by mercury is entered. The stopcock is closed, evacuation made to the 3 ml. mark, and then returned to the 0.12 mark after 1 minute shaking. The pressure (P_4) is read. The blank is determined by rinsing the instrument with water and then adding 0.1 ml. of ammonia buffer followed by 0.1 ml. of water and the mercury until the reagents are beyond the stopcock and the pressure read. Finally, 0.03 ml. of the cuprous chloride reagent is added as in the determination, and the second pressure read. The difference between the two readings is the blank to be used in the calculations.

Calculations

$$(P_3 - P_4 - \text{blank}) \times \text{factor (Table 4)} = \text{CO in vols./100 ml.}$$

$$\text{CO in vols./100 ml.} \times 0.446 = \text{CO in mM/l.}$$

Estimation of Iron in Whole Blood and Serum

Reagents

Iron Standard: 0.5 mg./ml. Add 100 ml. of distilled water to 3.51 Gm. reagent grade ferrous ammonium sulfate ($(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$). Add 10 ml. saturated potassium persulfate and dilute to 1 L. Stable at room temperature.

Saturated Potassium Persulfate: 8 Gm./100 ml. Stable at room temperature.

Sodium Thiocyanate (25%): Dissolve 25 Gm. NaSCN in a minimum of distil ed water. Add 2 ml. acetone and dilute to 100 ml. Store in refrigerator.

Perchloric Acid (70%): This analytical reagent contains 0.5–2.0 μg. Fe/ml.

Procedure

Iron in Whole Blood: 0.02 ml. of oxalated blood, or blood freshly drawn, is washed into 0.2 ml. of dist. H_2O. Use a constriction pipet (7) or an Accupipet[1] and a 13 × 100 mm. Pyrex culture tube which has been marked at 4 ml. Add 0.2 ml. conc. H_2SO_4 and 0.2 ml. 70% $HClO_4$.

[1] American Hospital Supply Corp., Evanston, Ill.

Digestion is carried out on a medium heated hot plate with tubes set at approximately a 15° angle to avoid bumping. The tubes are heated until clear. After cooling, approximately 1 ml. of H_2O and 0.2 ml. of saturated $K_2S_2O_8$ are added. After 1 minute, 0.4 ml. NaSCN solution is added and the mixture diluted to the 4 ml. mark with dist. H_2O. The reagent blank is similarly prepared.

For the standard, 0.02 ml. of the iron standard (10 μg.) is pipetted *from the same pipet* used for the blood. This cancels any pipet calibration error. If reaction is cloudy after cooling, it is due to incomplete digestion and is caused by precipitation of $KClO_4$.

The Klett-Summerson colorimeter with the #52 filter or the Coleman spectrophotometer (470 mμ) have been used for the readings.

Calculations:

$$\frac{\text{Reading of the unknown}}{\text{Reading of the standard}} \times 50 = \text{Fe mg./100 ml.}$$

Iron in Serum or Plasma: 0.5 ml. clear unhemolyzed serum or plasma is mixed with 0.5 ml. conc. H_2SO_4 and 0.5 ml. $HClO_4$ (70%) in a Pyrex tube (16 × 125). Digest on a medium heated hot plate until clear. 5 ml. H_2O and then 1 ml. NaSCN reagent are added and the solution mixed. 3 ml. colorless amyl alcohol is then added, mixing vigorously by hand for 30 seconds. After centrifugation the supernatant amyl alcohol layer is read in a colorimeter (#52 filter for Klett-Summerson, 495 mμ Coleman spectrophotometer).

The working standard is prepared by diluting the stock standard (500 μg./ml.) 1:250. 0.5 ml. (1 μg.) of the working standard is used. Distilled water in place of serum or plasma is used for the reagent blank.

The same procedure can be used for smaller samples. For 0.05 ml. serum aliquots using the Coleman spectrophotometer, Model 14, 3 ml. cuvettes with 5 cm. light path is used. 0.2 ml. aliquots can be assayed with the Klett-Summerson colorimeter using 2 ml., 4 cm. light path cuvettes. For these reduced amounts of sample, all reagents are reduced proportionally, except the amyl alcohol. 3.5 ml. is used with the Coleman cuvette and 2.5 ml. with the Klett-Summerson. The standard and reagent blank are also reduced accordingly.

In this determination the reagents used contain a significant amount of iron, making the reagent blank an important determination. To determine the amount of iron in the reagents read the color developed in the blank against colorless amyl alcohol. All reagents should be accurately measured.

Calculation:

$$\frac{\text{Optical Density of unknown}}{\text{Optical Density of standard}} \times 200 = \text{Fe } \mu\text{g./100 ml.}$$

This formula is the same for all quantities of serum or plasma.

RESULTS

The validity of the oxygen procedure was checked by comparing the results obtained for the O_2 capacity of the blood of 32 normal individuals with that of the iron content of the same blood. It has been established that the Fe to O_2 holding capacity of hemoglobin is 1 Fe to 1 O_2 (15) or 1:1 when both are expressed in mM/l. of blood.

In our experiments on normal adults we observed a range of 100–160 μg. of Fe/100 ml. of serum, which is in agreement with the reported values for plasma Fe (16). Since the whole blood Fe content is of the order of 55,000 μg./100 ml. it is apparent that the plasma Fe interference in the normal individual is outside the range of experimental error.

The values in the column for hemoglobin (Table 1) were obtained by the conventional colorimetric measurement (17). In this procedure the cells are hemolyzed in alkaline solution and the density of the color read. Cell debris, chromophoric substances other than hemoglobin present in blood such as bilirubin (particularly in the newborn), carotene, and lipids all serve to influence the reading. Further, an absolute standard is difficult to prepare in view of the instability of oxyhemoglobin (18). One usually prepares a standard curve based on iron or oxygen determinations. The standard curve used was the one supplied with the hemoglobinometer, as an outside reference. On the basis of the data in the O_2

Table 1. MEAN VALUES FOR BLOOD CONSTITUENTS FOR APPARENTLY HEALTHY ADULTS AND NEWBORNS

Subject	*Cases*	*Fe*[a] (mM/l.)	*Av. dev.* (±)	*O₂*[b] (mM/l.)	*Av. dev.* (±)	*Hemoglobin* (Gm./100 ml.)	*Av. dev.* (±)	*Hemoglobin*[c] *(Fe equiv.)* (mM/l.)	*Hematocrit value* (%)	*Av. dev.* (±)
Adult males	8	9.51	0.45	9.56	0.52	15.36	0.62	9.35	48.7	3.3
Adult females	8	8.28	0.18	8.17	0.53	13.10	1.04	7.98	41.8	3.3
Newborn males	8	10.01	0.78	9.97	1.07	16.01	2.49	9.75	54.4	6.6
Newborn females	8	9.49	0.74	9.67	0.63	15.89	1.10	9.68	50.4	4.8

[a] Fe, mM/l. × 5.585 = Fe, mg./100 ml.
[b] O_2, mM/l. × 2.24 = O_2, vol/100 ml.
[c] Hemoglobin value × 0.0609.

or Fe column this curve yields significantly lower values. The column showing the average deviation indicates a much broader range of values from individual to individual, than observed in the O_2 or Fe column. We believe this to be due to variations of these artifacts from individual to individual.

The hematocrit values (percentage red cells by volume) were estimated by a method previously described. They are only qualitatively related to the hemoglobin content in that the size and number of cells is not directly proportional to the hemoglobin content. The hemoglobin and hematocrit values both indicate roughly that the values obtained for O_2 are in the correct range. For convenience of comparison the hemoglobin values have been converted to "iron equivalent" by using the value 0.340 as the percentage of iron in hemoglobin (19).

Table 1 shows the results obtained in this study. These are divided into groups for adult males and females and newborn males and females. Wherever duplicate values for a particular determination differed by more than 3 per cent, the results were discarded except for O_2 capacity when a difference of 5 per cent in duplicates was accepted.

The columns for average deviation for each column are a measure of the variation from individual to individual. These columns are of interest in indicating the range of values to be expected in the particular group of individuals studied. To determine whether the O_2 method is valid it is necessary to demonstrate that, within the range of experimental error, the values obtained by the O_2 method are the same as those obtained by the iron method. The t test was therefore applied using the simplified procedure described by Varnum (20). The mean value for the Fe column, 32 cases, is 9.323. For O_2 it is 9.343. The variance (σ^2) for the Fe column is 0.3468 and the variance for the O_2 column is 3.3267, the sum of the variances being 3.674. Applying this to the chart of Varnum one obtains a necessary mean difference (D) of 0.023 to be exceeded for a significant difference of the means at the 5 per cent level. The actual difference of the means is 0.020.

While one would be justified in saying that the difference between the two columns is not convincing one cannot say that they are essentially identical. There is a suggestion that the oxygen values tend to be high. This may be due in part to oxygen dissolved in the plasma. Calculating the percentage difference between the Fe and O_2 values obtained for each of the 32 cases and averaging the percentage difference, one obtains a mean difference of 1.2 per cent with a standard deviation of ± 0.33. This seems to indicate more clearly that within the range of the experimental error of the methods the values for O_2 and Fe are in agreement.

Table 2. VALUES FOR BLOOD CONSTITUENTS IN THREE ANOXIC PREMATURE INFANTS AND ONE CASE OF SICKLE CELL ANEMIA

Case	*Diagnosis*	*Fe* (mM/l.)	O_2 (mM/l.)	*Hemoglobin* (Gm./100 ml.)	*Hematocrit value* (%)
1	Prematurity (anoxic)	8.33	8.66	12.9	45
2	Prematurity (anoxic)[a]	8.42	8.62	12.3	42
3	Prematurity (anoxic)	8.52	8.63	12.9	48
4	Sickle cell anemia	5.70	6.12	8.6	23

[a] Expired at 48 hours of age, lungs poorly expanded.

Table 2 lists the values obtained on the blood of 3 premature infants selected because they exhibited periods of cyanosis. The hematocrit values are lower than those usually observed, probably because these infants were receiving fluids parenterally. A case of untreated (not transfused) sickle cell anemia is included in the table. These cases are of interest in that the premature is claimed to have large amounts of fetal hemoglobin (5) and that certain types of anemias are characterized

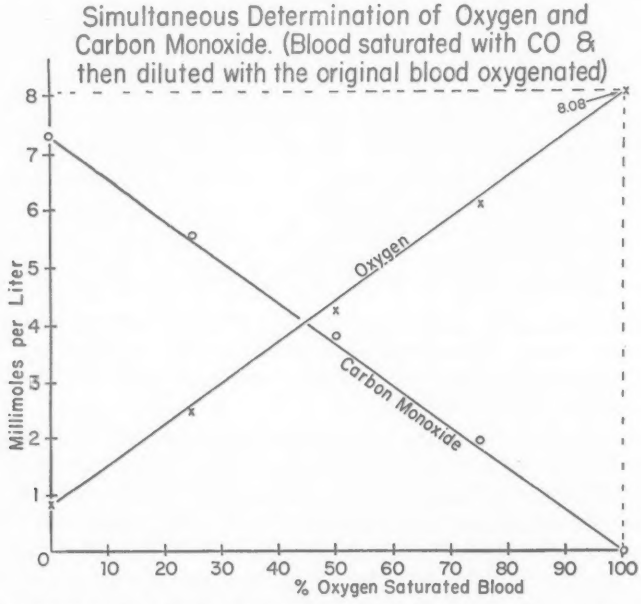

Fig. 4. Determination of oxygen and carbon monoxide content on the same sample with the microgasometer.

Table 3. Comparison of Percentage Oxygen Saturation of Fingertip and Arterial Blood

	Fingertip blood[a]	*Arterial blood*
O_2 Content (vol.%)	16.7	17.8
O_2 Capacity (vol.%)	20.1	20.3
O_2 saturation (%)	83	88

[a] Patient six years after operation for tetralogy of Fallot.

by the presence of a "fetal type" hemoglobin (21, 22). The O_2 capacity obtained is somewhat high for the amount of Fe found in the blood. Fetal hemoglobin has been crystallized and characterized as differing from hemoglobin of the normal adult (5). While this may be related to the differences observed, one must also consider the possibility that the lower hematocrit values, especially in the case of sickle cell anemia, with the higher proportion of plasma, may have exaggerated the effect of the dissolved O_2. The number of cases, is not adequate for statistical interpretation. It can be said, however, that in these 4 cases the hemoglobin did not exhibit a lowered ability to hold O_2.

Figure 4 shows the results obtained on blood saturated with CO and then analyzed for O_2 and CO content simultaneously, as it was diluted with blood from the same individual saturated with O_2. The points shown are compared with theoretical lines drawn from the original and final composition. Each point represents an average of four determinations. The distance of the points from the theoretical lines drawn indicates the expectable error.

The question is often raised as to whether the percentage O_2 saturation of blood taken from the fingertip is a true measure of the percentage O_2 saturation of arterial blood. Actually the results were always lower

Table 4. Factors for Calculating Oxygen or Carbon Monoxide Concentration in Volumes/100 ml.

Temp. (°C.)	*Factor*	*Temp.* (°C.)	*Factor*
18	0.494	27	0.478
19	0.492	28	0.476
20	0.490	29	0.475
21	0.488	30	0.473
22	0.486	31	0.472
23	0.485	32	0.470
24	0.483	33	0.468
25	0.481	34	0.467
26	0.480	35	0.465

in our series on fingertip blood but not by a consistent percentage when the arterial blood of the same individual was studied. A typical case is listed in Table 3. The lower values obtained from finger puncture are probably indicative of the fact that while fingertip blood is largely arterial blood, there is sufficient contamination from venous blood to render the results invalid for the accurate estimation of the percentage saturation of arterial blood. Air contamination is not the problem it would seem to be since the results obtained from fingertip blood were consistently lower than that obtained from arterial blood.

Fingertip blood percentage O_2 saturation values are useful, however, in following the course of certain individual conditions. For example, in premature infants, marked changes take place as the infant goes into or comes out of a condition of cyanosis. Because of the ease of obtaining fingertip blood practical use may be made of this procedure, if allowance is made for its limitations.

SUMMARY

Improved gasometric methods of analyses for oxygen and carbon monoxide in fingertip blood are described. The accuracy of the method for oxygen is evaluated by comparison with the iron concentration of the blood. The accuracy of the method for carbon monoxide is evaluated by analyzing samples of blood saturated with carbon monoxide serially diluted with the same blood saturated with oxygen.

Technics are described for obtaining the blood for analysis from fingertip puncture.

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Effect of Shaking on the Accuracy of Salt Fractionation Methods For Serum Albumin

Abraham Saifer and Michael C. Zymaris

A COMMONLY USED PROCEDURE proposed by Kingsley, (1), for separating the serum globulins from the soluble albumin fractions after salt fractionation, is shaking with ether and centrifugation. Among the salts most frequently used for the separation and determination of serum albumin are sodium sulfate (2), sodium sulfite (3), and mixtures of the two (4).

It has long been recognized that many protein solutions can be "denatured" by prolonged shaking at an air interface (5, 6) and this phenomenon is accelerated when such solutions are shaken in the presence of certain salts, such as sodium chloride or magnesium sulfate (6, 7, 8). Denaturation is defined for purposes of this discussion as the loss of solubility of albumin.

Several investigators (4, 9, 10) have stated that the shaking of albumin solutions in the presence of ether and of the salt used for the precipitation of the serum globulins (e.g., sodium sulfite) may lower the albumin values obtained. Wolfson *et al.* (9, 10) proposed the use of ether containing suitable surface-active agents (e.g., Span 20) for the centrifugal separation of globulins without alteration of the albumin values obtained. However, none of the above investigators have presented quantitative data on the conditions influencing serum albumin denaturation. It is the purpose of this paper to supply the experimental data required to eliminate this potential source of error for the accurate determination of serum albumin, α-globulins, and A/G ratios.

The results obtained by the salt fractionation technic described here,

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under conditions *least* favorable to albumin denaturation, compared closely with those determined electrophoretically for 42 different sera from both healthy subjects and patients.

EXPERIMENTAL

Reagents and Apparatus

1. Ether, reagent grade.
2. Ether-Span reagent. A mixture of 1 ml. of Span 20[1] with 99 ml. of ether is filtered through Whatman #1 filter paper and made up to the original volume with ether. It should be kept cold.
3. Sodium sulfite, 28%.
4. Biuret reagent (11).
5. Sodium sulfate, 23%. Store at 37°.
6. Sodium-sulfate–sodium-sulfite solution (4).
7. Albumin standard. A 25 per cent solution of human serum albumin[2] is diluted with 0.85% NaCl to give a concentration of about 5 per cent. The solution is then dialyzed overnight in a cellophane bag against running tap water. After dialysis, the solution is brought back to its original volume and enough NaCl is added to bring the salt concentration to 0.85 per cent. Total nitrogen and nonprotein nitrogen were determined by the Kjeldahl nitrogen method. The value obtained was used as the standard for all subsequent albumin determinations. The standard is preserved with a few crystals of thymol and remains stable for a 6-month period or longer if kept refrigerated.

Determination of Albumin with the Ether-Span Procedure

The chemical procedure described in this paper is essentially that used by Wolfson and Cohn (10) for the separation of human albumin using 26.9% sodium sulfite. The electrophoretic fractionation of human serum was performed with the portable Aminco electrophoresis apparatus using the technic previously described (12). The salt fractionation method is as follows:

Pipette 12.0 ml. of 28% sodium sulfite into a 15 mm. × 125 mm. o.d. test tube. Add 0.5 ml. of the standardized albumin solution (or serum) and mix the contents thoroughly by inversion. Then add 2 ml. of ether-Span reagent. Stopper the test tube and mix twenty times by gentle

[1] Atlas Powder Co., Wilmington, Del.
[2] Red Cross, Squibb & Son, N. Y.

inversion. (The tube is turned upside down and then back to its original position with stopper upright for *one* inversion.) Remove the stopper and centrifuge for 10 minutes at 2000 rpm. If the centrifugate is not perfectly clear, repeat the separation step with more ether-Span.

The precipitated globulins are packed at the ether–water interface in the form of a button. By carefully tilting the tube, the protein button disengages itself from the side of the tube permitting the withdrawal of a 5 ml. aliquot of the centrifugate. Biuret reagent (5 ml.) is then added to 5 ml. of the sample and the contents of the tube are mixed thoroughly by inversion.

After a 30 minute incubation period, the colors are read in a photoelectric colorimeter using a 540 mμ filter, adjusting the zero reading with a reagent blank which is carried through the procedure using 0.5 ml. of 0.85% NaCl in place of the albumin standard or serum.

A comparative study of the albumin values obtained with the electrophoretic and chemical methods for both normal and pathologic sera was performed and the results obtained are shown in Table 1.

Effect of Time of Shaking Using Tubes of Various Sizes

1. Wolfson-Cohn Procedure (10) with Pure Albumin

The procedure described above was carried out with 0.5 ml. aliquots of the standard albumin solution. The tests were run, in duplicate, in tubes of the following three sizes: 15 mm × 125 mm. o.d.; 16 mm. × 150 mm. o.d.; and 20 mm. × 150 mm. o.d. After all the components were introduced, the free air space in each tube is that shown in Fig. 1A. The tubes were shaken in one of two ways, either by gentle inversion, or by vigorous manual shaking for the indicated lengths of time. The effects on the albumin values are shown in Fig. 1A.

The exact procedure as described above was repeated using ether in place of ether-Span. The results obtained in these runs are given in Fig. 1B.

Table 1. Comparison of Albumin Results Obtained with Chemical and Electrophoretic Methods for Normal and Pathologic Sera

	Albumin (Gm./100 cc.)					
	Electrophoretic data			*Wolfson-Cohn procedure with ether-Span*		
	Mean	*S.D.*	*Range*	*Mean*	*S.D.*	*Range*
18 Normal sera	4.22	±0.36	3.68–4.92	4.10	±0.23	3.75–4.58
24 Pathologic sera	3.45	±0.66	1.50–4.39	3.47	±0.71	1.50–4.70

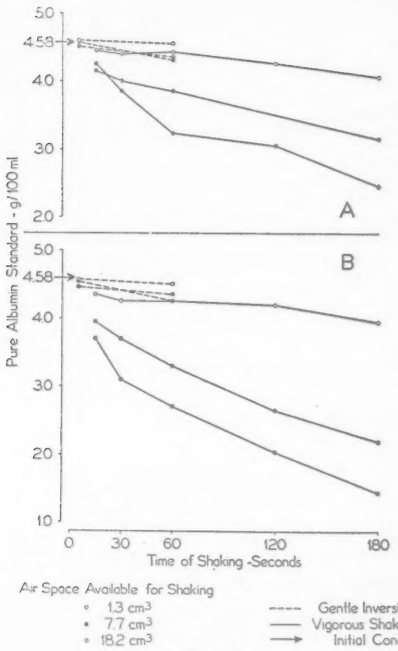

Fig. 1.

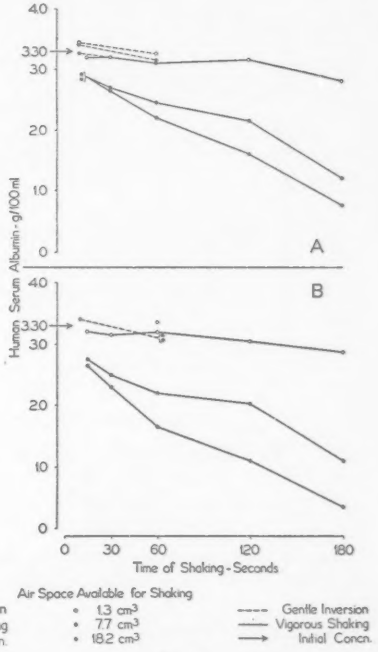

Fig. 2.

Fig. 1. *A*, Effect of shaking time on a 4.58% pure albumin standard in 26.8% Na_2SO_3 solution separated with the aid of ether-Span in tubes of various sizes. *B*, Effect of shaking time on a 4.58% pure albumin standard in 26.8% Na_2SO_3 solution separated with the aid of ether in tubes of various sizes.

Fig. 2. *A*, Effect of shaking time on a 3.30% human serum albumin (measured by electrophoresis) in 26.8% Na_2SO_3 solution separated with the aid of ether-Span in tubes of various sizes. *B*, Effect of shaking time on a 3.30% human serum albumin in 26.8% Na_2SO_3 solution separated with the aid of ether in tubes of various sizes.

2. Wolfson-Cohn Procedure with Serum Albumin

The procedures employing both ether and ether-Span were run as described in above, except that a pooled serum sample of known albumin content (as determined electrophoretically) was used in place of the albumin standard. The results are shown in Fig. 2.

3. Reinhold Procedure (4) with Serum Albumin

The albumin content of the same pooled serum samples was determined with the Reinhold procedure, except that tubes of different sizes were

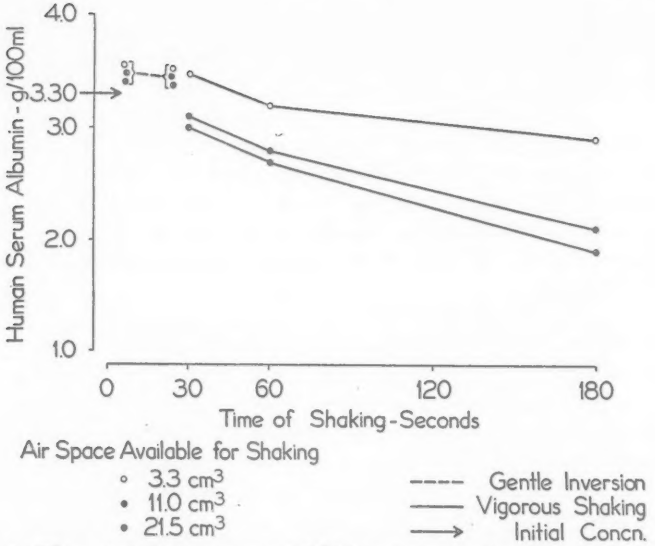

Fig. 3. Effect of shaking time on a 3.30% human serum albumin in 19.5% Na_2SO_4–6.6% Na_2SO_3 solution (4) separated with the aid of ether in tubes of various sizes.

used and the contents were shaken with ether for the length of time indicated in Fig. 3 instead of the 20 seconds recommended in the original procedure.

4. Pure Albumin, Serum Albumin, and Human Serum with 21.2% Na_2SO_4

The procedure used was the same as that described by Wolfson and Cohn (10) for the estimation of albumin plus α-globulins and human serum, except for the variation in shaking times and tube sizes noted in Figs. 4A and 4B.

RESULTS

Wu and Ling (6) showed experimentally in 1927 that the degree of denaturation obtained by shaking an egg albumin solution is markedly increased with time or with an increase in the size of the container. The magnitude of this effect for solutions of standardized pure human albumin in 26.8% sulfite is shown for the 3 minute shaking time in Fig. 1 where there is a decrease of about 44 per cent for ether-Span and about 67 per cent for pure ether below the initial albumin concentration. As is shown in Fig. 2, the magnitude of the denaturation effect for the albumin con-

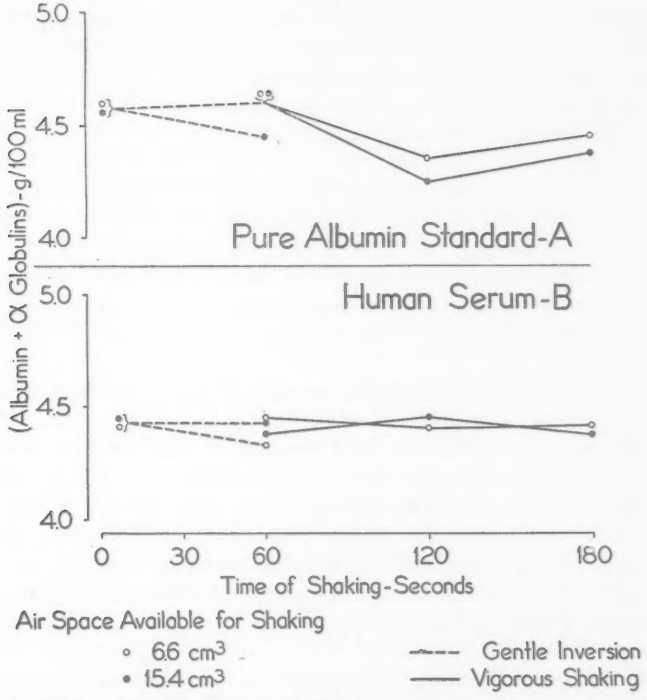

Fig. 4. *A*, Effect of shaking time on a 4.58% pure albumin standard in a 21.2% Na_2SO_4 solution separated with the aid of ether in tubes of various sizes. *B*, Effect of shaking time on human serum albumin plus alpha globulins in a 21.2% Na_2SO_4 solution separated with the aid of ether in tubes of various sizes.

tent of pooled human serum is even greater than that for the pure albumin solution—a 77 per cent decrease for ether-Span and a 88 per cent decrease for pure ether. The exact albumin concentration of the pooled serum sample was determined by electrophoretic analysis.

The data in both Figs. 1 and 2 indicate that the denaturation effect can be reduced both by decreasing the time of shaking and by using gentle inversion in place of vigorous shaking for any given size tube. The amount of denaturation can be further reduced to a relatively insignificant value by decreasing the space available for shaking to a minimum. In this latter case even a 3 minute period of vigorous shaking decreases the albumin concentration by less than 15 per cent. For the small tube, with a 1.3 $cm.^3$ free air space, gentle inversion of less than 30 seconds will reduce the denaturation error to 2 per cent or less—within the ex-

perimental error of the biuret method itself. The main advantage of ether-Span over ether as a reagent for the rapid centrifugal separation of albumin from the precipitated globulins, is the enhanced ability of this reagent to effect this process with a minimum of shaking and not its small protective action against denaturation.

The data in Fig. 3 show that the albumin content of the pooled human serum sample, as determined with the sodium-sulfate–sodium-sulfite reagent of Reinhold (4), decreases about 44 per cent after shaking 3 minutes in the largest size tubes. Again, as in the case of the Wolfson-Cohn procedure (10), this decrease can be reduced to within the experimental error of the photometric method by employing small tubes with a free air space of 3.3 $cm.^3$ or less and by gentle shaking for less than 30 seconds.

The data in Fig. 4 indicate that albumin denaturation does not occur to any appreciable extent with ether shaking in the presence of 21.2 per cent sodium sulfate with either an increase in the time of shaking or with increase in the tube size. This holds true whether a pure albumin solution (as in Fig. 4A) or human serum albumin is used (as in Fig. 4B). This fact is of importance in the accurate determination of the total α-globulin content of serum with the Wolfson-Cohn procedure (10) as this is determined as a difference of two large numbers so that any slight error in the albumin procedure will introduce a significant error in the α-globulin values.

With proper precautions with respect to tube size, the use of ether-Span and with a minimum of gentle shaking, the values obtained with the Wolfson-Cohn procedure (10) give mean values for both normal and pathologic sera which check with the electrophoretic method. As can be seen from the data in Table 1, the two methods check with an average error of about 3 per cent for both normal and pathologic sera. The mean value obtained for 18 normal sera with the chemical method is 4.10 gm./100 cc. with a standard deviation of $\pm.23$. The mean value for the same 18 sera as determined electrophoretically is 4.22 gm./100 cc. ± 0.36 S.D. Since the chemical albumin values were determined in duplicate on two separate aliquots of serum, the dispersion from the mean as measured by the standard deviation is somewhat less for this method than for the single-run electrophoretic procedure.

DISCUSSION

Excellent comprehensive review articles on the subject of protein denaturation have been published by Neurath *et al.* (13), by Anson (14, 15), and by Putnam (16). Protein denaturation has been defined (13, 16)

as any nonhydrolytic change of the unique structure of native proteins, giving rise to definite changes in chemical, physical, or biologic properties, such as decreased solubility. Wu (17) proposed that the phenomenon be looked upon as a change from the unique and specific rigid structure of the native protein to the much more randomly arranged denaturated form. Anson (15), while stating that no rigid definition of denaturation is possible at present, suggests that the process involves the breaking of a considerable number of looser bonds within the molecule, i.e., hydrogen bonds, salt bonds, and van der Waals bonds, making the protein more like a simple peptide chain. While the term "denaturation" may be divided experimentally into three distinct steps—denaturation, flocculation, and coagulation—it has been employed loosely in this presentation to include all three stages resulting in the loss of albumin solubility.

Numerous studies have appeared in the literature with regard to protein denaturation by means of heat, concentrated acids and bases, alcohol, urea, guanidine salts, alkyl sulfates, x-rays, ultraviolet radiation, surface actions, etc. (13–17), but only those of Ramsden (5) and Wu and Ling (6) have dealt directly with protein denaturation during the shaking process. The latter investigators have made especially careful studies on the effects of surface active substances, salts and nonelectrolytes, *p*H, and temperature on the rate of coagulation during the shaking process. Their experimental findings show that shaking egg albumin solutions in the presence of ether *decreases* the amount of coagulation and that the presence of high concentration of sodium chloride or foaming of the protein solutions has little influence on the denaturation of albumin.

Denaturation by shaking results from "rolling up" the surface film and unlike heat denaturation cannot be separated into distinct stages. The effect of temperature on the rate of coagulation by shaking of human serum albumin has not been studied in the present investigation. However, Wu and Ling (6) have shown that for egg albumin the temperature coefficient for a 10° rise (25° to 35°) is only 1.09. Changes of this magnitude are seldom encountered in the laboratory and would be largely offset by their simultaneous effect on both standards and unknowns as well as the cooling effect due to increased ether evaporation at higher temperatures.

Our own experimental findings with respect to human serum albumin have confirmed, in general, those of Wu and Ling (6) for egg albumin. It was found that albumin denaturation was caused mainly by prolonged shaking in the presence of high concentrations of sodium sulfite, whereas

ether alone had no effect, while solutions of sodium sulfate exerted a protective action as previously reported by Boyer, Ballou, and Luck (18).

While previous investigators (4, 9, 10) have given due attention to the time of shaking as a potential factor for albumin denaturation, little stress has been placed on the importance of the available air space as a cause of protein denaturation. When proper precautions are taken with respect to *tube size* as well as the technic of shaking, results are obtained for serum albumin with either the Wolfson-Cohn (10) or Reinhold (4) method which check closely with the more expensive and time-consuming electrophoretic method.

Accurate albumin values are not only important clinically in a wide variety of disease states (19) for both diagnostic and prognostic purposes, but are especially important where it is desired to determine accurately other protein fractions—such as α-globulins—by means of salt fractionation technics.

SUMMARY

1. The important factors causing albumin denaturation in the presence of sodium sulfite when shaking with ether or ether-Span, are the method and time of shaking, and the free air space available for shaking.

2. Gentle inversion with ether-Span for 30 seconds or less, and a free air space of about 1.3 cm.^{3} gave results with the Wolfson-Cohn procedure (10) which were in close agreement with the electrophoretic method in 42 normal and pathologic sera.

3. There is little or no albumin denaturation from shaking with ether in the presence of 21.2% sodium sulfate as used in the procedure of Wolfson-Cohn (10) for albumin plus α-globulins.

ACKNOWLEDGMENTS

The authors wish to acknowledge the aid of Dr. Bruno W. Volk, Director of Laboratories, who permitted part of the experimental work in this paper to be performed under Grant No. B-285 of the U. S. Public Health Service dealing with the general subject of "Protein Studies in Chronic Diseases." We also wish to thank Miss Renee Eisner for editing and typing the manuscript.

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Convenient Apparatus for the Continuous Dehydration of Histologic Material

Guy Nadeau and L. P. Joly

USUAL METHODS OF DEHYDRATING histologic material, such as repeated passages through weak to strong solutions of alcohol, are rather time consuming, and the time lost in the recovery of the solvent has compelled most laboratories instead to discard it after use. Such a practice can amount to enormous expense of material. Automatic devices reduce to a minimum the personnel and the time required for their operation, but they can hardly be afforded by small organizations.

The apparatus (Fig. 1) described in this paper was devised at the request of the Department of Pathology and is an example of how the special technics of a chemistry department may be used advantageously to solve problems of other laboratory sections.

The method and apparatus involve the continuous extraction of water from specimens by ethanol which, in turn, is dehydrated by distillation over quicklime. Soxhlet extractors cannot be used with advantage since in such apparatus the specimens are immersed in hot solvent and may be readily coagulated. This can be avoided, however, by having the solvent cooled before it reaches the samples.

COMPONENTS

The apparatus, which may be held to a single stand with the aid of extension clamps, consists mainly of the following parts:

1. One 2-liter, 3-necked, round-bottom flask. This flask is placed far enough from the base of the stand to allow easy dismantling and cleaning without having to move the rest of the assembly.
2. An ascending column of about 22 mm. O.D., insulated with asbestos

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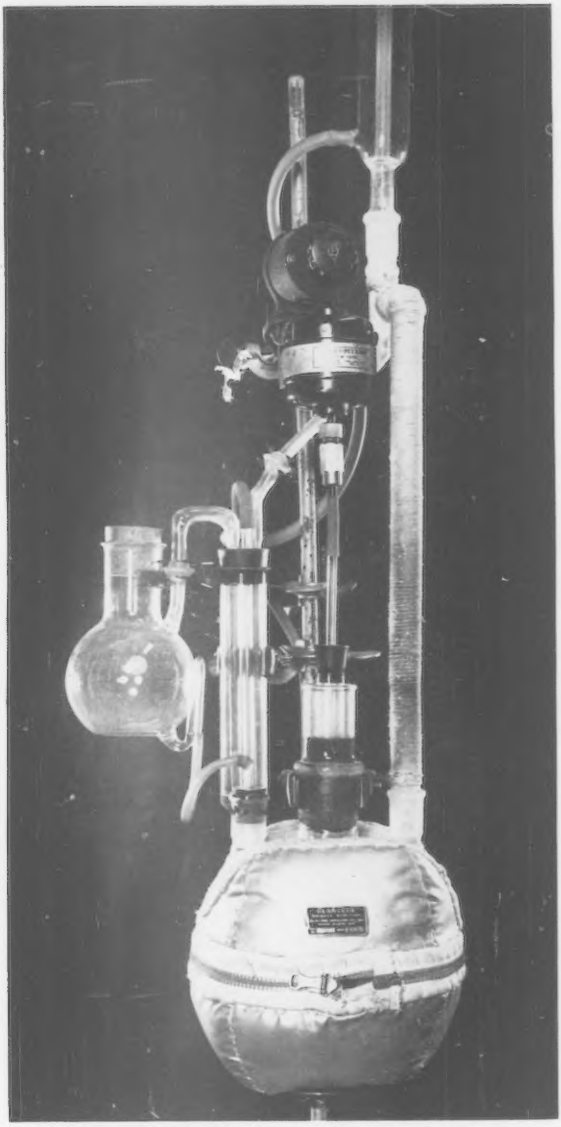

Fig. 1. Apparatus for continuous dehydration.

thread to reduce the loss of heat by radiation and increase the rate of distillation.

3. An efficient condenser for the ethanol vapors. The top inlet is protected against air moisture with a calcium chloride tube (not shown in illustrations).

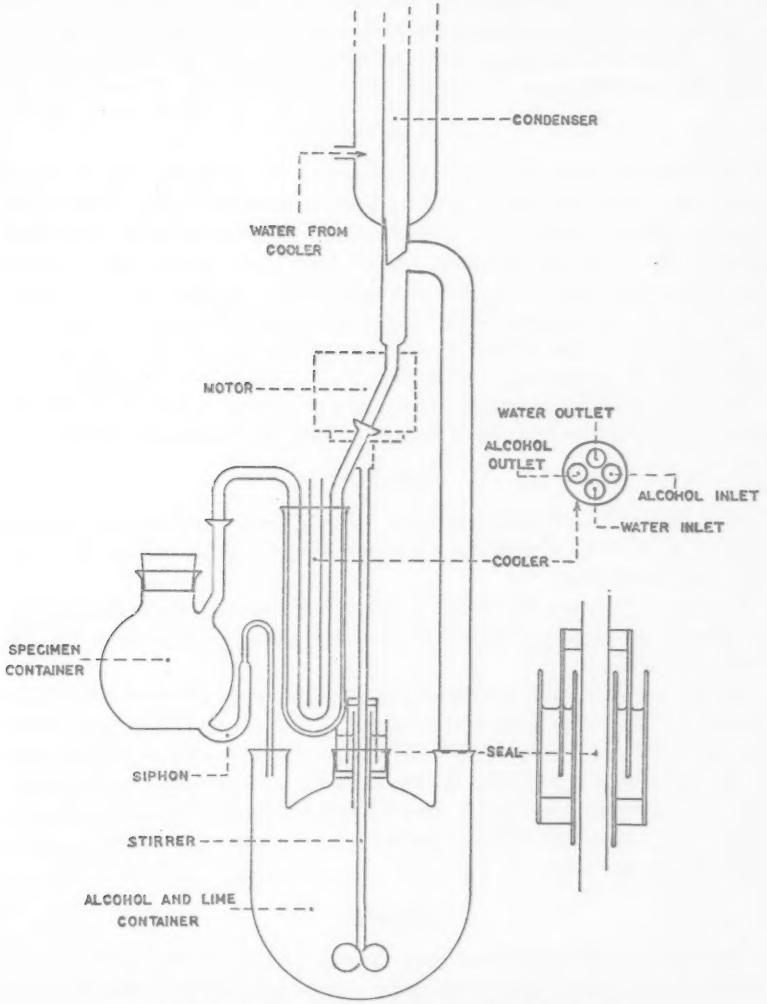

Fig. 2. Diagram of assembly and manner of operation.

4. A cooler built from a large test tube (about 35 mm. O.D.), a U-shaped tube for the solvent, an inlet and an outlet for the water, the former reaching the bottom of the device.
5. One 500-ml., wide-mouth specimen container, to which has been added a 12-mm. O.D. glass inlet and a 5-mm. O.D. glass outlet siphon. (The ratio of both diameters should be as described to avoid undue suction of the solvent from the cooler). It should be kept in mind that the height of the siphon will regulate the rate of renewal of solvent in the container.

Additional Features

An efficient stirrer is added to eliminate the bumping due to solid quicklime. It should be actioned by a variable speed motor. Glass seals may be obtained from various manufacturers or conveniently assembled (Fig. 2). Mercury was first used, but on account of unavoidable spattering and vapor formation, was advantageously replaced by a viscous, alcohol-insoluble polymer (equal parts of Indopol H-25 and H-100).

Distillation of the solvent is effected with an electric heating mantle (Glas-Col). A supporting ring is placed under the whole apparatus.

The use of ball-and-socket type joints (See Figs. 1 and 2) in the assembly reduces the hazards of breakage through torsion or vibration.

OPERATION

The 3-necked flask is lowered and 1.2–1.3 liters of ethanol are added, with about 50 gm. of quicklime (calcium oxide, lumps, technical) to insure smooth stirring.

To avoid bumping, the stirrer is started as soon as the heating mantle is plugged in. More power will be required at the beginning to stir the sludgy mass.

Small specimens may be individually wrapped in gauze for further identification. The interval of time allotted for each bath will depend upon the rate of distillation of alcohol and the height of the siphon (20 minutes in the apparatus described). It can be easily adjusted after a few trials. The time necessary for proper dehydration will be set by experience, but it has been found that a 2-hour period is quite sufficient for biopsy specimens of average size.

CLEANING

The lime has to be replaced from time to time, according to the rate of turnover of material. Most of the pure solvent may be recovered from the

disconnected siphon in another container. The 3-necked flask is lowered and washed thoroughly with water (hydrochloric acid will dissolve any residue), followed by absolute ethanol.

COMMENT

This compact (12″ × 12″ × 25″ without condenser), inexpensive, and easily built device has now been in constant use for nearly a year without mechanical failure. It has reduced the time necessary for the dehydration of specimens to one fourth, and the expense of ethanol to one fifteenth, of the previous figures.

the Clinical Chemist

MEMORANDUM

SUBJECT: Recognition of Specialists in Other Fields

FROM: Dr. Edward L. Turner, Secretary
Council on Medical Education and Hospitals
American Medical Association
535 North Dearborn Street
Chicago 10, Illinois

DATE: June 18, 1954

At the December, 1953, meeting of the Board of Trustees of the American Medical Association, the Committee on Specialists in Other Fields indicated that it believed the subject of the relationship of American medicine to professional nonmedical groups in the health field was of such importance that a small *ad hoc* committee could not do justice to the problem. The Committee recommended that the Board of Trustees and the Council on Medical Education and Hospitals and its staff, in association with the appropriate councils and bureaus of the American Medical Association and relevant professional groups undertake a detailed investigation of all facets of the problem and that following the receipt by the Committee of a report from the Council on its findings and recommendations, the Committee on Specialists in Other Fields will submit its recommendations to the Board of Trustees. The Board concurred in this recommendation of the Committee and reported to the House of Delegates in December, 1953. Following adoption of the report by the House of Delegates, the Board of Trustees voted to request the Council on Medical Education and Hospitals to study this matter and to report back to the Board at the earliest opportunity as to a possible plan of procedure, the recommendation of the Committee including personnel needs, cost, and so on.

The Council on Medical Education and Hospitals has been making a careful analysis of the various facets of this problem and recognized the need for aid from allied professional organizations. An expression of

This memorandum is being published with the consent of Dr. Edward L. Turner.

opinion on the part of various medical organizations will be of great assistance to the Council in formulating the recommendation it should prepare for the Committee on Specialists in Other Fields. The following material has been prepared in order to present the general trends and to seek your counsel and advice.

The rapid increase in knowledge over the past three decades has led to the necessity for the development of many subgroups or specialty areas in what were formerly covered by larger general fields of interest in medicine. For example, in the field that was once just medicine we now recognize, besides the general internist, allergists, neurologists, psychiatrists, dermatologists, gastroenterologists, cardiologists, chest specialists and other experts. Similarly, in the surgical area there are now such specialties as thoracic surgery, urology, neurosurgery, plastic surgery, proctology, ophthalmology, otolaryngology, and orthopedics in addition to the general surgeon. Even in some of the narrower specialty areas such as radiology, we now see further subdivision such as has been brought about by the recognition of diagnostic roentgenology and therapeutic radiology.

In some of the specialty areas in other fields essential to adequate modern diagnosis, prevention, and therapy, similar increments of knowledge have been taking place which have necessitated varying degrees of specialization and widespread recognition of the need for such developments.

The area of microbiology may be utilized as a specific example of one of the fields that has seen great advances during recent years in its various disciplines such as (a) virology, (b) mycology, (c) bacteriology, (d) parasitology, and (e) immunochemistry. The rate of progress in this over-all field has been tremendous, and the field of microbiology in general is a very broad one, encompassing many other disciplines than medicine. Medical microbiology, on the other hand, has a unique place in the diagnosis, prevention, and therapy of disease, with all its areas of concern constantly assuming roles of ever-greater importance.

In order to qualify as a competent medical microbiologist it is necessary that an individual have the broad background and training of general microbiology essential to adequate understanding of this field. Since in order to obtain such training it is necessary that a potential medical microbiologist spend a long period of time working toward a degree that will give him authoritative recognition in his field, the question of the Ph.D. degree versus the M.D. de-

gree assumes considerable significance and brings up the main reason for focusing attention on this problem at the moment.

An increasing number of specialty areas closely allied to medicine and requiring the equivalent of Ph.D. training are either seeking or have already sought some form of recognition or endorsement through the development of independent American boards or subspecialty boards in their fields. Suggestions have been made in regard to the establishment of a new American Board of Microbiology and an American Board of Medical Microbiology in recent years. In the course of these efforts, certain problems have been encountered in regard to the possible relationship of such boards with medicine. The problem now confronting us is whether a mutually satisfactory solution to this situation can be developed that might well serve as a pattern of possible future developments in specialty areas requiring a similar high standard of training for eligibility as a specialist.

Using the microbiologists as an example again, it should be pointed out that if a microbiologist devotes adequate time to his basic training in this field so that he can qualify as a specialist in medical microbiology, the time spent in completing the requirements for a Ph.D. degree is essentially the equivalent of that required in obtaining an M.D. degree. Although there are certain obvious advantages for a medical microbiologist to have a full M.D. background in addition to his Ph.D. training, the question may be raised as to whether in the area of diagnosis which he covers the former degree in itself is or is not essential to the task he must assume. Actually, much of the time spent in certain phases of preparatory work qualifying for the degree of M.D. would be essentially wasted time for the individual who specifically plans to be a medical microbiologist. The same problem confronts those who might be primarily concerned with areas such as biochemistry. In view of the increasing time demands for training in all areas today, there seems to be a very real need for recognition of the possibilities of conservation of time in preparation for activities in various special fields.

The biochemist, the psychologist, the biophysicist, the health educator, and others requiring Ph.D.-type training, and who are to become part of the over-all team approach to medicine today, fall into a category somewhat similar to that of the microbiologist, as has already been indicated.

It is of paramount importance that in the diagnosis and care of

patients the physician constantly remain the pivotal figure and that these various areas of activity allied to medical diagnosis, prevention, and therapy be as closely coordinated with medicine as possible. If such coordination is to be effectively accomplished there must be mutual understanding of the obligations of the various individuals representing these allied specialty fields to each other and to the patient. Such close cooperation can best be obtained through recognition of the responsibilities lying in the various areas of activity and through setting standards of the type that will render the best possible over-all end results from the standpoint of the welfare of the patients. The question may be raised, therefore, as to whether in light of the contributions which these qualified specialists in the areas associated with medicine make today in prevention, diagnosis, and therapy in cooperation with practitioners of medicine, they should or should not have some form of recognition or endorsement through recognized specialty or subspecialty boards, if their training and experience so indicates and providing they possess either an M.D. or Ph.D. degree or both. It is fairly obvious from the current trend of events that some of these groups have been seriously endeavoring to obtain endorsement for the development of such specialty or subspecialty boards during recent years. At the present time it is apparent that some will proceed to develop their own organizations in the relatively near future, with or without the sanction of the medical profession. The question can be raised as to whether it is more desirable to have these organizations proceed entirely independently or whether there might be certain mutual advantages through having close liaison with such groups and with medicine in general.

Actually, the questions that are presented in this situation seem to be relatively simple, but are deserving of very serious consideration at this time when there are constantly increasing efforts being made to set up boards in these various fields related to medical activities. Among these questions are the following:

1. In view of the importance of these specialties in other areas in the prevention, diagnosis, and treatment of disease, do you believe that the medical profession has a responsibility in assisting in developing the standards to be maintained by them?
2. If the medical profession has a responsibility in this regard, how can it best be assumed?
3. Should the Council on Medical Education and Hospitals of

the American Medical Association and/or certain other interested groups endeavor to assist in the development of certifying boards or agencies which would establish and maintain standards mutually acceptable to the Council and the specialty group?

4. What in your opinion is the best form of cooperative or liaison relationship between the medical profession and these various specialized groups?

Your cooperation in giving consideration to this problem and in sharing your views with the Council on Medical Education and Hospitals will be of great value in helping arrive at a solution in the best interest of all concerned.

EDWARD L. TURNER, M.D.

COMMITTEE ON CLINICAL CHEMISTRY

The Committee on Clinical Chemistry of the American Chemical Society met at the Netherlands Plaza Hotel, Cincinnati, Ohio, on April 1 and 2, 1955. The main topics under discussion dealt with legislation as it pertains to clinical chemistry. John G. Reinhold, past president of the Association, was present as Chairman of the committee.

NEW EDITOR OF *STANDARD METHODS*

Dr. David Seligson, director of the Division of Biochemistry, The Graduate Hospital, University of Pennsylvania, was appointed editor-in-chief of *Standard Methods for Clinical Chemistry*, a laboratory manual sponsored by the Association and published by the Academic Press, New York. The first volume appeared in 1953, and Volume II is now in preparation. Dr. Seligson's appointment on March 15, 1955, is for a five-year period.

NEW SECTIONS

Greater Kansas City

The Executive Committee of the American Association of Clinical Chemists has approved a Greater Kansas City Section. The charter date for this section was February 16, 1955.

Texas

The Executive Committee of the American Association of Clinical Chemists has approved a Texas Section. Temporary officers appointed at the organizational meeting were: *President pro tem*, Major J. A. Rivera of Fort Sam Houston; *Secretary pro tem*, Dr. J. W. Goldzieher of San Antonio. The charter date for this section was April 8, 1955, and is the ninth local section to be established.

REPORTS FROM THE SECTIONS

Metropolitan New York

A. Saifer

A symposium on bone and calcium metabolism was held on Tuesday, April 26, 1955, at Blumenthal Auditorium of the Mount Sinai Hospital. Franklin C. McLean of the University of Chicago spoke on "Homeostatic Regulation of Calcium Ion Concentration in Blood." Daniel Laszlo, Montefiore Hospital, presented "New Tools for the Study of Mineral Metabolism in Man." Albert E. Sobel, Jewish Hospital of Brooklyn, was symposium chairman. A general discussion followed the formal presentations.

The following slate of officers was elected for the year 1955: *Chairman*, Julius Carr, The Mount Sinai Hospital; *Vice-Chairman*, Harry Goldenberg, Hillside Hospital; and *Secretary-Treasurer*, Abraham Saifer, Jewish Chronic Disease Hospital. Members of the *Executive Committee* are Charles L. Fox, Jr., I. J. Greenblatt, Alexander I. Greenstein, Bernard Klein, Mary H. McKenna, and Kurt G. Stern.

Washington-Baltimore-Richmond

Marian E. Webster

One of the largest exhibits devoted exclusively to medical research instruments in this country was held at the National Institutes of Health, Bethesda, Md., May 2–5, 1955, under the sponsorship of the manufacturers whose products were exhibited.

A symposium on "Recent Developments in Research Methods and Instrumentation," sponsored by the Washington-Baltimore-Richmond Section of the Association, the Washington Section of the American Chemical Society, the Instrument Society of America, and the Society of American Bacteriologists was held concurrently.

Topics scheduled for the symposium sessions included "Electrophoresis and Chromatography," "Fluorescence Determination," "Optical Methods in Biochemistry," "Selection of Electronic Components for Medical Research," and "Instrumentation for Cytochemical and Microbiological Research."

There was an interesting demonstration of new apparatus, with many of the exhibited instruments in actual operation. The exhibit, which has been an annual event for the past five years, attracted a wide representation of research workers and other personnel from medical installations, pharmaceutical houses, universities, and hospitals in various parts of the country, as well as visitors from the many research installations in the Washington area.

The third meeting of the 1954–55 year was held on March 3, 1955, at the National Institutes of Health. A symposium on recent advances in hematologic technics

was presented by Lt. Col. Joseph H. Akeroyd, who discussed the development and recent advances in the Coomb's test; Dr. William H. Crosby, Jr., who commented on the difficulties encountered in the standardization of methods for clinical hemoglobinometry and suggested the use of the Drabkin technic; Naomi Benjamin, who discussed the methods, errors, and reason for determination of red cell indices; and Dr. William A. O'Brien, who reported on the various technics for the determination of blood volumes.

Code of Ethics of the American Association of Clinical Chemists, Inc.

Approved and Passed by the National Executive Committee, September, 1953

ARTICLE I: DEFINITIONS AND GENERAL CONSIDERATIONS

What Constitutes Clinical Chemistry

Section 1

Clinical chemistry is that branch of chemistry which deals with the composition of the secretions, excretions, concretions and fluids of the human body in health and disease, and the chemical composition and metabolism of cells and tissues. Also the search for the presence of substances (or their derivatives) given for diagnostic or therapeutic reasons and the search for poisons (or their derivatives) are properly included in the field of clinical chemistry.

What Constitutes a Clinical Chemist

Section 2

Any individual equipped by education and experience to engage in the practice of clinical chemistry as defined above shall be considered a clinical chemist.

Responsibility of the Clinical Chemist

Section 3

The profession of clinical chemistry, as an adjunct to the profession of medicine, has as its ultimate responsibility the welfare of the public. The clinical chemist shall use to the best of his ability his scientific skills and knowledge to the benefit of all men without regard for racial or religious origin.

Education and Experience

Section 4

The clinical chemist shall have as his goal the acquisition of the best available education and experience in chemistry. He shall strive to constantly enlarge and improve his knowledge.

Relationship to the Medical Profession

Section 5

The clinical chemist shall deal with the medical profession at all times at the highest professional level. The compensation by the patient for chemical services shall include no rebates or commissions to any persons for solicitation or referral of analyses.

Relationship to the Patient

Section 6

The clinical chemist shall perform no services to the patient except on advice or prescription from any licensed practitioner of the medical arts. All reports and discussion of chemical findings shall be only between the chemist and the physician in charge.

ARTICLE II: PUBLICATION, PATENTS, AND ADVERTISING

Dissemination of Scientific Information

Section 1

The clinical chemist shall freely discuss with his fellow chemists and with scientists in related fields, advances in the science of clinical chemistry. To withhold information for personal gain shall be considered unethical. This Section shall not apply to information classified by a government agency for reasons of national security.

Publication of Research Findings

Section 2

An obligation to publish, after critical evaluations, new knowledge pertaining to the science of clinical chemistry obtained through research or other observations, shall be acknowledged.

Advertising and Publicity

Section 3

The clinical chemist shall not use, or allow his name to be used, in advertising directed to the public. Professional announcements shall be brief, dignified and consistent with accepted customs in medical and allied fields. The clinical chemist shall not seek publicity, yet he shall recognize the right of the public to have access to information concerning the public health and welfare. Publication of a scientific article or book

shall precede the release of such material to the lay press. Because of the danger of misinterpretation, he must use restraint and great caution in releasing information having diagnostic or therapeutic implications.

Patents and Commissions

Section 4

The application of discoveries and developments in clinical chemistry, directly effecting public health and welfare, should not be limited by unreasonable restrictions for personal gains to the clinical chemist.

ARTICLE III: OBLIGATIONS AS A CHEMIST

Accuracy of Chemical Analyses

Section 1

The clinical chemist shall have as his goal the attainment of the highest precision and specificity that existing procedures permit.

Reporting of Significant Figures

Section 2

The analyst shall not report figures or decimal places that lack significance.

Critical Survey of Methods

Section 3

It shall be considered inadequate practice for a clinical chemist to use any procedure that has not been adequately studied in his own laboratory.

Replicates and Recoveries

Section 4

The systematic use of controlled procedures, such as replicates and recoveries, shall be considered indispensable to good practice.

Results of Uncertain Magnitude

Section 5

The clinical chemist shall not report any result of uncertain magnitude of error, unless this uncertainty is clearly made known to the recipient of the report.

ARTICLE IV: INTERPRETATION OF RESULTS

Relationship to the Physician

Section 1

The clinical chemist shall, at the request of the physician in charge of the patient, outline to the physician the significance of any chemical findings, and suggest further determinations that would aid the physician in making a diagnosis or prognosis. The clinical chemist shall under no circumstance transmit to the patient either the results or the interpretation of the results. The clinical chemist shall receive no compensation from the patient for interpretation of results to the physician.

ARTICLE V: THE CLINICAL CHEMIST AS AN INDIVIDUAL

A Scientist at All Times

Section 1

The clinical chemist shall conduct himself as a scientist at all times.

High Regard for Medical Profession

Section 2

The clinical chemist shall hold in high esteem the profession of medicine, to which he is an adjunct.

Relationship with Analysts

Section 3

The clinical chemist shall carefully supervise the analysts working in his laboratory. He shall train these workers to the best of his ability, encourage them to attain the highest professional competence, and teach them by word and example to adhere to the ethical standards herein outlined.

Publications and Collaborators

Section 4

The clinical chemist shall contribute as much as possible to research and advancement of his specialty. He shall encourage those working in his laboratory to do likewise. He shall accept as collaborators whenever possible the junior members of his staff and encourage these members to contribute to the science of clinical chemistry. He shall to the best of his

ability assist physicians and other scientists by fully collaborating in their efforts to advance medical science.

ARTICLE VI: CONCLUSION

The ethics of the clinical chemist shall at no time be inferior to the standards long prevailing in the medical profession. The outline here presented can act only as a general guide, and shall be periodically reviewed and revised. It is for the individual to judge his professional conduct in the light of his obligation as a scientist to serve mankind.

ABSTRACTS

Editor: ELLENMAE VIERGIVER. *Contributors:* JOSEPH A. ANNINO, GLADYS J. DOWNEY, CLYDE A. DUBBS, ALEX KAPLAN, MARGARET M. KASER, MIRIAM REINER, HERBERT THOMPSON

Determination of ethylenediaminetetraacetic acid (EDTA) as the chromium complex. P. J. Cherney, B. Crafts, H. H. Hagermoser, A. J. Boule, R. Harbin, and B. Zak. (*Wayne University, Detroit, Mich.*)

A simple, rapid and reliable determination of EDTA is presented which depends on measuring the light absorbence of its purple Cr^{III} complex. The high stability of this complex prevents interference from other ions, since Cr^{III} is in excess.

Two ml. of urine are pipetted into a cuvette. Two ml. of Na_2CrO_4 solution (5 Gm./l.) and 2 ml. of 0.2N H_3AsO_3 solution are added (9.89 Gm. H_3AsO_3 + 7 Gm. NaOH in 300 ml. of water, neutralize with glac. $HC_2H_3O_2$, + 400 ml. excess, and dilute 1:1). The solution is heated in a boiling water bath 5 minutes, cooled, and read at 560 mμ.

Considerable data on favorable recovery are given. The nature, formation, and stability of the EDTA-Cr^{III} complex are discussed. Complete spectrophotometric data are included.—*Anal. Chem.* **26,** 1806 (1954).

C. D.

Some errors in the determination of calcium in aged blood serum eliminated by flame photometry. P. S. Chen, Jr., and T. Y. Toribara (*University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*)

The calcium of aged (cloudy or partially denatured) serum, unlike that of fresh serum, cannot be quantitatively precipitated by oxalate, except after preliminary deproteinization with CCl_3COOH. Errors involved in the determination of Ca as CaC_2O_4 can be eliminated by direct Ca determination using the flame photometer. Very brief data are given.—*Anal. Chem.* **26,** 1967 (1954).

C. D.

Direct ethylenediamine tetraacetate (EDTA) titration methods for magnesium and calcium. C. W. Gehrke, H. E. Affsprung, and Y. C. Lee. (*Missouri Agricultural Experimental Station, Columbia, Mo.*)

An EDTA direct titration method, eliminating phosphate interference, has been used for 2 years for routine Ca determination in plant materials, milk, urine, serum, rations, grains, and other biologic materials.

A 10 Gm. sample of plant material is dry ashed at 550°, digested 2–3 hours with HCl-HNO_3, and diluted to 250 ml. Phosphate is removed by passing a neutralized 25 ml. aliquot containing 10–15 mg. of Ca through a column (23 × 2 cm. diameter) containing a 9 cm. zone of anion exchange resin, Amberlite IR-4B, Cl form, at a rate of 2–3 ml. per minute. The Ca is titrated in the combined eluate and washings with EDTA, using murexide indicator.

Considerable data indicate good Ca recoveries despite wide variations in phosphate concentration, and good agreement with and better precision than parallel results using the oxalate-permanganate method. Experience is necessary to detect the elusive purple end point. Magnesium determination is given for limestone only.—*Anal. Chem.* **26,** 1944 (1954).

C. D.

Rapid estimation of free and total cholesterol. B. Zak, R. C. Dickenman, E. G. White, H. Burnett, and P. J. Cherney. (*Detroit Receiving Hospital and Detroit Memorial Hospital, Detroit, Mich.*)

Simple and rapid procedures for total and free cholesterol determination in 1 ml. of serum are described. One milliliter of serum is placed in a 25 ml. volumetric flask containing about 10 ml. of acetone-alcohol (1:1) with swirling, after which the contents are heated to boiling in a water bath. After cooling, acetone-alcohol is added to 25 ml. and the contents are filtered. For total cholesterol, 2.5 ml. of filtrate in a 30 ml. test tube is evaporated to dryness. The residue is dissolved in 3 ml. of glac. $HC_2H_3O_2$ with heat. Two milliliters of color reagent [1 ml. of stock (2.5 Gm. $FeCl_3 \cdot 6H_2O$ in 25 ml. of glac. $HC_2H_3O_2$, stored in the refrigerator) in 100 ml. of conc. H_2SO_4] is added and the contents are mixed thoroughly. After cooling, the color is measured at 560 mμ. For free cholesterol, 2.5 ml. of filtrate in a 15 ml. conical centrifuge tube is evaporated to 0.5 to 1.0 ml. and 1 ml. of 1% digitonin in 50% ethanol is added. After 10 minutes, the tube is centrifuged for 10 minutes at 3500 rpm. and the supernatant liquid is removed by decanting. The precipitate is washed with 4 ml. of acetone and centrifuged. The acetone is decanted and the tube drained by inversion for several minutes on absorbent paper. The precipitate is dissolved in 3 ml. of glac. $HC_2H_3O_2$ with heat, 2 ml. of color reagent is added, and the color intensity measured as for total cholesterol.

A standard curve is prepared by subjecting 0.1, 0.2, and 0.3 ml. aliquots of a cholesterol standard (1 mg./ml. in glac. $HC_2H_3O_2$) to the color reagent.

Recovery experiments are shown as well as simultaneous analyses by the Kingsley-Schaffert procedure for total cholesterol [*J. Biol. Chem.* **180,** 315 (1949)] and by a modified procedure involving the Liebermann-Burchard reaction. Recoveries and correlations with the procedures mentioned are good. The sensitivity of this procedure is given as approximately 5 times that of the Schoenheimer-Sperry.

The abstracter concurs with the authors' conclusion that the simplicity, speed,

accuracy, and reproducibility of the method may well recommend it for routine use.—*Am. J. Clin. Pathol.* **24,** 1307 (1954).

H. E. T.

Effect of rest and activity on the serum protein fractions. T. P. Whitehead, A. P. Prior, and D. F. Barrowcliff (*South Warwickshire Hospital Group (14), Warwick, England*)

Total serum protein, albumin, alpha, beta, and gamma globulin, hemoglobin, and hematocrit were determined on 15 normal persons after several hours of rest and after a similar period of normal activity. After activity there is a significant and consistent rise in total serum protein, with a mean increase of 0.47 Gm. per 100 ml., due almost entirely to an increase in the albumin fraction. No significant changes in average globulin, hemoglobin, and hematocrit were observed.—*Am. J. Clin. Pathol.* **24,** 1265 (1954).

H. E. T.

Coagulation studies in abruptio placentae complicated by hypofibrinogenemia. T. J. Greewalt and D. C. Triantaphyllopoulos. (*Milwaukee Blood Center, Milwaukee, Wisc.*)

Low fibrinogen levels and hemorrhagic manifestations may occur in abruptio placentae, amniotic fluid embolism, or with prolonged intrauterine retention of a dead fetus. The escape of thromboplastin-rich material causing in vivo defibrinogenemia within the maternal circulation may be the pathogenesis common to all these syndromes, although activation of fibrinolytic systems has been a suggested mechanism [Weiner *et al., Am. J. Obst. & Gynec.* **66,** 475 (1953)]. Levels of fibrinogen and labile factor (Ac-globulin) and prothrombin consumption and thromboplastinogen activity time were determined on 4 patients with premature separation of the placenta. All patients had fibrinogenopenia with fibrinogen below 0.2 mg./100 ml., and prolonged prothrombin times by the one-stage method, which were shown to be due to a deficiency of labile factor. Studies of prothrombin consumption and thromboplastinogen activity offer some suggestion of the presence of platelet factor deficiency and perhaps low thromboplastinogen levels also.—*Am. J. Clin. Pathol.* **24,** 1246 (1954).

H. E. T.

Studies on the excretion of chloride by man with and without congestive heart failure, using long-life radiochloride, Cl^{36}. C. T. Ray, S. A. Threefoot, G. E. Burch. (*Tulane University School of Medicine, New Orleans, La.*)

Daily excretion patterns of Cl^{36} in urine and stool were studied for prolonged periods after a single intravenous administration of 50 μc. of the tracer. The concentrations of Cl^{36}, Na, and K were determined in serum, urine, and other body fluids. Stools were assayed for Cl^{36} only. The method of Schales and Schales

was used for Cl determination. From the fact that subjects with congestive heart failure are not able to excrete administered NaCl as readily as control subjects and the noted observation of the relationship of the immediate excretion of Cl^{36} and body weight, it is suggested that electrolyte excretory function tests might be developed as indicators of cardiorenal function in congestive heart failure.—*J. Lab. Clin. Med.* **44**, 663 (1954).

G. J. D.

The estimation of Darstine 1-(3-hydroxy-5-methyl-4-phenylhexyl)-1-methylpiperidinium bromide and its tertiary analog, 5-methyl-4-phenyl-1-(1-piperidyl)-3-hexanol hydrochloride in plasma and urine. E. K. Tillson, S. F. Paulson, and K. H. Beyer. (*Merck & Co., Inc., West Point, Pa.*)

The method described depends on the fact that the drugs can be extracted into ethylene dichloride from an alkaline aqueous medium. The solvent layer is shaken with an aqueous solution of methyl orange and the colored salt formed at the interface, which is soluble in ethylene dichloride, is acidified and read colorimetrically. A method for differentiating the two drugs depends on the fact that chloroform extracts the tertiary compound but not Darstine from an aqueous solution.—*J. Lab. Clin. Med.* **44**, 880 (1954).

G. J. D.

A new method for analysis of plasma fibrinogen utilizing a cationic detergent. R. F. Jacox. (*University of Rochester School of Medicine, Rochester, N. Y.*)

A turbidimetric technic for analysis of fibrinogen utilizes the property of fibrinogen to react with a cationic detergent, octadecyl dimethyl benzyl ammonium chloride (Octab), at a low *p*H and a high NaCl concentration. Fibrinogen is precipitated from a 1 ml. sample with sat. $(NH_4)_2SO_4$ and redissolved to a volume of 5 ml. with 1% NaCl. To 0.1 ml. of this solution is added 8.9 ml. of phosphate-NaCl buffer (0.1M Na_2HPO_4: 0.1 M NaH_2PO_4 with 0.4M NaCl; *p*H 5.1) and 1 ml. of Octab, 1% aqueous. At 15 minutes optical density is read with a 440 mμ filter against standard human fibrinogen. The accuracy of the procedure is ± 6 per cent. Values by this method are high compared with a clotting method of fibrinogen assay, due to the fact that lysed fibrin as well as unaltered fibrinogen reacts with the reagent.—*J. Lab. Clin. Med.* **44**, 885 (1954).

G. J. D.

Separation of the bile pigments of serum, bile and urine. P. G. Cole, G. H. Lathe, and B. H. Billing (*University of London, London, England*)

The pigments of serum and urine of jaundiced patients and of bile were isolated by a combination of extraction procedures and reverse phase chromatography.

The absorption curves of the isolated pigments before and after diazotization were obtained in the ultraviolet and visible range. The nonpolar bile pigment that predominates in the serum of patients with hemolytic jaundice was bilirubin. The serum of patients with obstructive jaundice contained two pigments that reacted "directly" in the van den Bergh test. The visible absorption curves of the azo compounds of these three pigments are indistinguishable.—*Biochem. J.* **57,** 514 (1954).

A. K.

Quantitative estimation of glucose by paper partition chromatography. S. Baar. (*Birmingham Accident Hospital, Birmingham, England*)

Paper chromatograms were prepared of solutions containiug 50–200 μg. of glucose, using a *n*-propanol-ethyl acetate-water system. The glucose was stained by drawing the dried chromatogram through a solution consisting of isopropanol, phthalic acid, and aniline. After drying, the stained areas were cut out, eluted with glac. $HC_2H_3O_2$, and the glucose estimated quantitatively by measuring the light absorption of the eluate at 480 mμ. The recovery of glucose varied from 97.0 to 102.0%.—*Biochem. J.* **58,** 175 (1954).

A. K.

A colorimetric estimation of sugars using benzidine. J. K. N. Jones and J. B. Pridham. (*The University, Bristol, England*)

A new colorimetric method for the determination of aldopentoses, aldohexoses' hexuronic acids, methylated sugars, and disaccharides is described. The sugar solution is heated at 100° for prescribed periods of time, depending upon the sugar, with a solution of benzidine in glac. $HC_2H_3O_2$. The resulting color is read at 425 mμ. From 20 to 600 μg. of aldopentoses and aldohexoses may be determined with an accuracy of ±3 per cent.—*Biochem. J.* **58,** 288 (1954).

A. K.

The determination of *N*-methyl-2-pyridone-5-carboxamide in human urine. J. M. Price. (*University of Wisconsin, Madison, Wisc.*)

N-methyl-2-pyridone-5-carboxamide (pyridone) appears in urine as a metabolite of nicotinamide. Interfering substances are removed by passing the urine through cation and anion exchange resins. The effluent is then analyzed directly for pyridone by measuring its ultraviolet absorbence in a spectrophotometer. Recoveries are within 95 to 100.1%. Of 16 closely allied substances tested, only *N*-methyl-2-pyridone-3-carboxamide was found to interfere.—*J. Biol. Chem.* **211,** 117 (1954).

J. S. A.

The quantitative determination of fructose with skatole and hydrochloric acid. B. M. Pogell. (*Brooke Army Medical Center, Fort Sam Houston, Texas*)

To equal volumes of sample and 0.04% skatole in a colorimeter tube are added 8 ml. of ice-cold 30% HCl. The tube is heated for exactly 40 minutes at 37° and the optical density of the purple color formed is read at 510 mμ against a water blank. The method follows Beer's law closely and has a precision of ±1%. —*J. Biol. Chem.* **211,** 143 (1954).

J. S. A.

Studies on uric acid and related compounds: 1. Quantitative determination of uric acid in biological fluids. F. Bergmann and S. Dikstein. (*Hebrew University Hadassah Medical School, Jerusalem, Israel*)

Uric acid may be determined in urine or plasma by deproteinization with $HClO_4$, precipitation of the uric acid with $Hg(C_2H_3O_2)_2$, redissolving the uric acid in an NaCl solution and measuring absorbency at 290 mμ. The method requires at least 2.5 ml. of plasma. It is specific even in the presence of xanthines and substituted uric acids.—*J. Biol. Chem.* **211,** 149 (1954).

J. S. A.

Formazan formation compared to phosphatase activity in human serum. A. L. Waldo, R. E. Zipf, and P. Burton. (*Miami Valley Hospital, Dayton, Ohio*)

Two milliliters of 2,3,5-triphenyl-2-H-tetrazolium chloride (Eastman) in phosphate buffer, *p*H 7.2, were incubated with 0.2 ml. of serum in the dark at 38° for 24 hours. The tubes were cooled in an ice bath, 8.0 ml. of acetone were added, and the tubes shaken. The formazan formed was determined by measuring the color intensity at 485 mμ with the Beckman Model B spectrophotometer. Alkaline and acid phosphatase activities were determined by the methods of Bodansky and Gutman. Similar values were obtained for patients with cirrhosis, Cushing's syndrome, renal rickets, hypo- and hyperparathyroidism, and neoplasms. The greatest color intensities were found in cancer of the prostate and osteogenic sarcoma. Further investigation disclosed a marked correlation between serum phosphatase activity and formazan formation.

The authors were unable to confirm the report of Straus *et al.* [*Science* **108,** 113 (1948)] and of Black *et al.* [*Am. J. Path.* **26,** 1097 (1949)] that malignant tissues stain more deeply with tetrazolium salts than do normal tissues.—*Proc. Soc. Exp. Biol. Med.* **87,** 19 (1954).

M. M. K.

Blood, urine and tissue amylase in depancreatized rats. J. H. Roe, Jr., B. W. Smith, and C. R. Treadwell. (*George Washington University School of Medicine, Washington, D. C.*)

Eight adult rats were depancreatized by a technic previously described [*Proc. Soc. Exper. Biol. & Med.* **86,** 878 (1954)]. Twenty-four-hour urine specimens and tail blood samples were collected at intervals up to 30 days after pancreatectomy. No differences in blood or urinary amylase levels as determined by the method of Smith and Roe [*J. Biol. Chem.* **179,** 53 (1949)] were found between the depancreatectomized rats and a comparable group of unoperated animals. Analyses of the various organs showed amylase to be present in similar amounts in all organs except the small intestine and the liver. The lower values for the intestine after operation can be explained by the absence of pancreatic secretions. The decrease in liver amylase may have been due to the development of fatty livers in the operated rats. The data suggest that some organ or organs other than the pancreas is the source of blood, urinary, and tissue amylase in normal rats.—*Proc. Soc. Exp. Biol. Med.* **87,** 79 (1954).

M. M. K.

Vitamin B_{12} serum levels and diabetic retinopathy. B. F. Chow, D. A. Rosen, and C. A. Lang. (*John Hopkins Hospital, Baltimore, Md.*)

Serum vitamin B_{12} was determined by the method of Okuda, Wood, Lang, and Chow [*Fed. Proc.* **13,** 1546 (1954)]. Previous work has shown that following the intramuscular administration of a test dose of vitamin B_{12}, diabetics with retinopathy excreted significantly more of the vitamin than nondiabetics, and diabetics without retinopathy excreted less than nondiabetics.

In the present study there were 18 diabetics without and 16 diabetics with retinopathy. The mean serum vitamin B_{12} level of the group without retinopathy was 162 ± 18 $\mu\mu$g. per ml., and for the retinopathic group 292 ± 24. With the exception of one subject in the non-retinopathic group the two groups could be separated by a dividing line at 200 $\mu\mu$g./ml. The difference between the two groups was statistically significant, with $p < 0.001$.

These data suggest that vitamin B_{12} is intimately involved not only in the metabolism of carbohydrate but also plays a fundamental role in the development of retinopathy.—*Proc. Soc. Exp. Biol. Med.* **87,** 38 (1954).

M. M. K.

Response of serum inorganic phosphate to insulin in normal and diabetic subjects. F. De Venanzi, M. Roche, and J. Vera. (*Instituto de Investigaciones Médicas, Fundación Luis Roche, Caracas, Venezuela*)

Twenty diabetic patients were divided into two groups on the basis of the response of serum phosphate to the intravenous administration of 1 ml. per kg. of body weight of 50% glucose. At 45 minutes Group I (12 subjects) had a phosphate fall of less than 0.30 mg. per 100 ml., and Group II had a decrease of more than 0.30 mg. per 100 ml. Insulin in a dosage of 0.1 unit per kg. of body weight was given intravenously to 42 normal young adults and to the diabetic subjects, all of whom were fasting and kept at rest for one half hour before and

during the test. Venous blood samples collected before and 12, 30, 45 and 60 minutes after the injection of insulin were analyzed for glucose by the Nelson method and for serum inorganic phosphate by the procedure of Fiske and Subba-Row. The mean decreases in blood sugar of the normal subjects were 39 mg. per 100 ml. at 15 min., 43 at 30 min., 32 at 45 min., and 18 at 60 min. The corresponding decreases for all the diabetics were 24, 39, 53, and 53 mg. per 100 ml.

All the differences between the normal and diabetic subjects were statistically significant, and only the initial blood sugar levels of 196 and 154 mg. per 100 ml. for Group I and Group II, respectively, of the diabetic subjects differed significantly.

The mean fall in serum inorganic phosphorus in the normal subjects was 0.53 mg. per 100 ml. at 15 min., 0.95 at 30 min., 0.99 at 45 min., and 0.75 at 60 min. For the diabetics these values were 0.29, 0.39, 0.51, and 0.33 mg. per 100 ml. All the differences between the normal and diabetic subjects were significant, and there was no significant difference between the two groups of diabetics.

The data indicate an inhibition of the response of phosphate to insulin in diabetes and that diabetics who differ in their serum-phosphate fall after glucose may be alike in the response of phosphate to insulin. It is believed that the phosphate fall after glucose reflects, at least in part, the ability of the pancreas to secrete insulin and that experimental data thus far available do not permit relating it to "peripheral utilization" of carbohydrate.—*Proc. Soc. Exp. Biol. Med.* **87,** 16 (1954).

M. M. K.

Determination of melanocyte-stimulating hormone in urine and blood. K. Shizume and A. B. Lerner. (*University of Oregon Medical School, Portland, Ore.*)

A procedure is described for the extraction of melanocyte-stimulating hormone (MSH) from urine and from blood. The concentration is assayed by determining the darkening of isolated frog skin with a reflection meter. Recovery studies showed that approximately 20 per cent of MSH is recovered from urine and approximately 60 per cent from blood.

The concentration of MSH in urine and blood is the same for normal males and females. The 24-hour urinary excretion values range from 7.5 to 47.5 units, with an average of 27.7 units. In blood the range is 0.4–2.2 units/ml., with an average value of 1.3 units. During pregnancy, MSH excretion becomes elevated at the end of the second month and continues to increase until term. Following delivery, there is a return to normal within a few days. Seven of 12 patients with Addison's disease and 4 out of 8 patients who had bilateral adrenalectomies excreted increased quantities of MSH. Some of these patients may have excreted normal amounts because they were receiving cortisone. The MSH excretion was elevated in 1 patient each with one the following conditions: new lentigines, alopecia areata totalis, and retinitis pigmentosa. Negroes, albinos, and patients with vitiligo showed normal MSH excretion values. Two of 8 patients with de-

creased pituitary function excreted decreased amounts of MSH. Administration of cortisone to a patient with high levels of MSH in blood and urine reduced the hormone concentration to normal. Following adrenalectomy the blood MSH level increased markedly in a dog and returned to normal when cortisone was administered.—*J. Clin. Endocrinol. & Metab.* **14,** 1491 (1954).

M. R.

Renal function and I^{131} clearance in hyperthyroidism and myxedema. C. J. Hlad and N. S. Bricker. (*Fitzsimons Army Hospital, Denver, Colo.*)

Renal function studies have been performed on 4 patients with hyperthyroidism, 5 with primary myxedema, and 2 with panhypopituitarism and probably secondary hypothyroidism. Glomerular filtration rate, renal plasma flow, and TmPAH (maximum capacity of the tubules to transport *p*-amino hippurate) values were elevated in the hyperthyroid group and depressed in the myxedematous group. Following therapy in 1 hyperthyroid patient, renal functions were reversed toward normal. Following long-term therapy in 1 myxedematous patient, the glomerular filtration rate increased despite a fall in renal plasma flow. Triiodothyronine administered intravenously to an untreated myxedematous patient for a period of 6 days induced a striking increase in renal glomerular filtration rate and renal plasma flow.

It has been concluded that the state of thyroid activity exerts a definite effect on renal hemodynamics and tubular function. The clearance of I^{131} in hyperthyroid and myxedematous patients has been found to be a linear function of the glomerular filtration. The state of thyroid activity seems to influence the renal excretion of iodide indirectly through a filtration-rate effect, but not at the tubular level.—*J. Clin. Endocrinol. & Metab.* **14,** 1539 (1954).

M. R.

The diagnostic use of I^{132}. E. M. Hanbury, J. Heslin, L. G. Stang, W. D. Tucker, and J. E. Rall. (*Sloan-Kettering Institute, New York, N. Y.*)

The application of I^{132}, with a half-life of 2.33 hours, to previously designed tracer technics is presented. Clinical data on 11 patients studied with I^{132} include 1-hour thyroidal uptake determinations and 30-minute thyroidal clearance rates. $Iodine^{132}$ would seem to find its chief merit in tracer experiments in which minimal radiation is desired and in experiments in which frequent measurements of iodine uptake is desired.—*J. Clin. Endocrinol. & Metab.* **14,** 1530 (1954).

M. R.

The mechanism of endocrine control of melanin pigmentation. A. B. Lerner, K. Shizume, and I. Bunding. (*University of Oregon Medical School, Portland, Ore.*)

The melanocyte-stimulating hormone (MSH) from the pituitary gland causes darkening of human skin and nevi and formation of new nevi. When large doses

of MSH were given to 1 patient, increased pigmentation was observed within a few hours. When MSH was given intramuscularly, from 1 to 4 per cent was excreted in the urine. After intravenous administration approximately twice as much was excreted.

The action of other hormones is related to that of MSH. Hydrocortisone and cortisone probably inhibit the release of MSH from the pituitary gland. Noradrenaline and adrenaline can block the local action of MSH on melanocytes, the pigment cells. The first factor is of clinical significance because cortisone and hydrocortisone can prevent the development of hyperpigmentation in patients who have undergone bilateral adrenalectomy and in patients who have Addison's disease. It can also decrease pigment already present in Addison's disease. The action of adrenaline and noradrenaline, although potent in vitro, has not yet proved to be of clinical significance. In addition, progesterone, which causes darkening of frog melanocytes, may have an effect on human melanocytes. Melanocyte-stimulating hormone seems to be a distinct pituitary hormone. Pigmentation associated with the clinical administration of ACTH is probably due to MSH contamination.—*J. Clin. Endocrinol. & Metab.* **14,** 1463 (1954).

M. R.

Effect of caloric restriction on cholesterol atherogenesis in the rabbit. M. G. Goldner, L. Loewe, R. Lasses, and I. Stern. (*Jewish Hospital, Brooklyn, N. Y.*)

Adult male rabbits were fed a diet of commercial rabbit pellets supplemented with raw carrots. For Experiment A, 18 animals were given 1 Gm. of cholesterol daily; 9 of these received a ration of pellets approximately half the average daily consumption of the 9 controls, which were fed *ad libitum*. In Experiment B, 22 animals were used. The food consumption of the 11 in the experimental group was decreased to one third that of the 11 controls, and the dose of cholesterol was increased to 2 Gm. daily for both controls and experimental animals. The durations of the two experiments were 12 and 8 weeks, respectively.

At intervals the following analyses were done: total blood cholesterol by the method of Abell *et al.* [*J. Biol. Chem.* **195,** 357 (1952)]; blood fatty acids by the method of Stern and Shapiro [*Brit. J. Clin. Path.* **6,** 158 (1953)]; lipid phosphorus by the procedure of Youngburg and Youngburg [*J. Lab. and Clin. Med.* **16,** 158 (1930)]; and lipoproteins according to Gofman *et al.* [*Circulation* **2,** 161 (1950)]. The two experiments gave similar results, with the experimental animals showing a loss of weight and a much more marked elevation of the blood lipid fractions than the controls. Arteriosclerotic changes with advanced lesions were present in the aortas of both groups and appeared to be more pronounced in the underfed rabbits. It is concluded that severe caloric restriction does not offer protection, but rather enhances the development of cholesterol-induced atherosclerosis in rabbits.—*Proc. Soc. Exp. Biol. Med.* **87,** 105 (1954).

M.M.K.

BOOK REVIEWS

Practical Clinical Biochemistry

HAROLD VARLEY. New York, Interscience Publishers, Inc., 1954, 558 pp., $6.50.

The author describes in 28 chapters procedures for all analyses required from a clinical chemistry laboratory, including clinical microscopy and the determination of the basal metabolic rate (Chapters VII and XX). The text contains 70 graphs and illustrations, almost all of very instructive character, also numerous tables and structural formulas in a little more than 500 pages of text. By painstaking compilation, the author has achieved a comprehensive description of technics, which include most of the more modern methods. In addition to the more commonly performed procedures, a large number, not usually found in routine manuals, are included, such as inulin and thiosulfate clearance tests, paper electrophoresis of serum proteins, choline esterase, serum copper, and pyruvic acid. Several alternatives for the analysis of a substance are frequently presented with the author's indications as to their individual usefulness.

The arrangement of the chapters is logical rather than systematic, as some chapters deal with groups of chemical entities, such as "The Plasma Proteins," "Chlorides, Sodium and Potassium," "Vitamins," "Hormones," while others deal with disease or dysfunction, for example, "Chemical Tests in Kidney Disease" and "Tests in Liver and Biliary Tract Disease." The same lack of pedantry is apparent in the organization of the subtitles. The description of every method is regularly followed by a few paragraphs with the author's extremely sound *interpretations* of the results. Each chapter concludes with a judiciously selected bibliography.

The prothrombin method described in Chapter XIV yields normal values of around 30 seconds rather than the usual figure of about 14 seconds, possibly owing to the selection of human brain rather than rabbit or horse liver as source for the thromboplastin. The instruments described for exemplification of photoelectric colorimetry and flame photometry (Chapters I and XVIII) are of British make. Besides such differences due to regional usage there are several points where this writer disagrees with statements and evaluations and where he misses some recent accessions to the armamentarium of clinical chemistry. This is of course to be expected in such a rapidly developing discipline, and Varley's book is altogether a most useful volume. Its strongest features are undoubtedly its comprehensiveness and the clear, unambiguous style in which technics and calculations are described.

The book will form an excellent basis for instruction in laboratory courses in routine chemistry for technicians and medical students. It is more than the

usual phrase of approval by a reviewer when we say that no clinical laboratory should be without it, although the text may be a little too much for junior technicians working without chemical direction, say, in a physician's office.

Mt. Sinai Hospital, N. Y. HARRY SOBOTKA

The Lipids: Their Chemistry and Biochemistry

Volume II.: Biochemistry (Digestion, Absorption, Transport and Storage)

HARRY J. DEUEL, JR. New York, Interscience Publishers, Inc., 1955, 919 pp., $25.00.

Dr. Deuel's work of the past eight years started out to encompass two volumes. Volume I, dealing with the chemistry of the lipids, is the most comprehensive work on the subject to date. Volume II, which was to include the biochemical data, had to be divided into two volumes as the past few years has seen a tremendous increase in investigations and published data in this fertile field of study. This first volume on the biochemistry of lipids discusses four topics, Digestion, Absorption, Transport, and Storage. The next volume will include Biosynthesis, Metabolism, Oxidation, and Nutritional Value.

Once again, as in the first volume, the author has done an excellent work in the organization of the subject. The chapter heads are broken down into a multitude of subheads and further divisions that bring the data into sharp focus for the reader. This interesting approach can be seen from the following example: Chapter II, *The Digestion and Absorption of Fats in the Gastrointestinal Tract*, is divided into 9 major heads. Heading 2, *Enzymes Concerned with the Digestion of Lipids*, has 3 subheads, Lipases, Esterases, and Other Lipid-hydrolyzing Enzymes. These subheads are further divided into the specific points of discussion so that there is a definite portion of the discussion devoted to a specific topic, as Cholinesterases in Blood, Cholinesterases in Brain and Nervous Tissue, etc. It is by strict adherence to this excellent outline of the subject that Dr. Deuel has provided a source book that does not scatter the subject and makes for ready rapid reference.

Under the author's presentation of blood lipids is found a most complete discussion of lipid alterations in disease and organ dysfunction. Animal experimentation studies as well as data from clinical medicine are presented. Parts of this discussion are carried further under another heading, Lipid Storage under Abnormal Conditions.

Lipid Distribution in Specific Tissues also discusses data from investigations concerned with animal material used as food, such as eggs and milk.

What has been said of Dr. Deuel's first volume can be repeated for Volume II. The book is well written. When one views the amount of material contained in its 919 pages, one realizes that the author has presented the subject matter without being verbose, yet has kept good continuity and reader interest. This work will greatly influence research in the field because in even casual reading

one can feel the author's ability to indicate to the reader just which parts of the data need further investigation and just where there is a definite lack of data, so that knowledge of a particular topic is sparse.

This book will save the researcher a tremendous amount of library work as the bibliography is most complete. The volume also contains a rather complete author, as well as subject index. There also is a listing of plant and animal sources of lipids.

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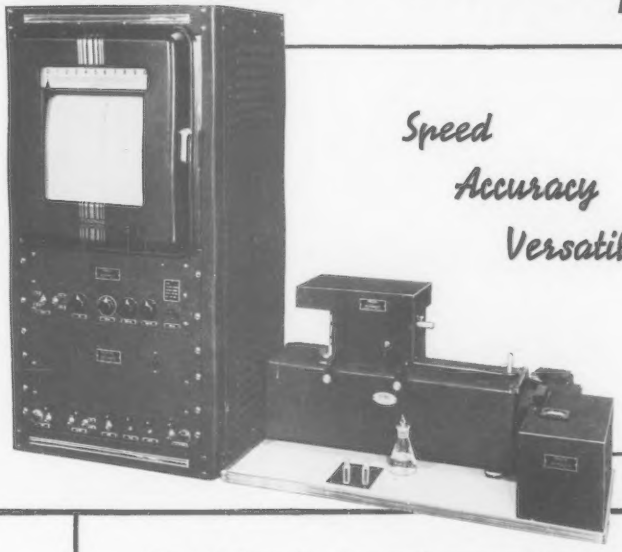

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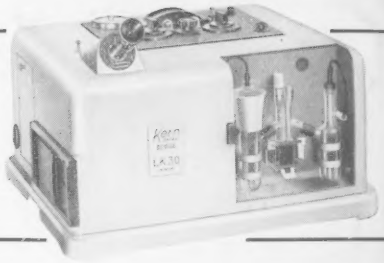

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